SAND AND WATER CULTURE METHODS used in the study of PLANT NUTRITION

by

E. J. Hewitt

Technical Communication No. 22 of the
Commonwealth Bureau of Horticulture and Plantation Crops
East Malling, Maidstone, Kent

COMMONWEALTH AGRICULTURAL BUREAUX

THIS book describes the methods devised to study the growth and mineral nutrition of plants in controlled cultures. Special attention is given to the problems of trace contamination and methods for the efficient study of the plant micronutrients. Containers, sand, water, nutrient composition and supply, aeration, biological factors and other matters are also considered in detail.

The study of plant nutrition is of fundamental and economic value, and much remains to be learnt. The modern techniques for sand and water cultures have developed mainly from the pioneer studies of Salm-Horstmar, Sachs and Knop.

Many of the twelve known essential mineral elements were recognised at the time of this early work. Others, e.g., molybdenum, were identified, often by chance, only lately owing to their almost ubiquitous distribution in the culture materials, in sufficient amounts to satisfy the plant's minute requirements.

It is hoped that the information compiled and the standard techniques described will serve as a guide and reference work for all who contemplate or practise the exacting yet fascinating study of plant nutrition in sand or water culture.

42/- or
$6.25

CAB INTERNATIONAL
00091798

P.R-D.

CABI BIOSCIENCE
EGHAM LIBRARY

SAND AND WATER CULTURE METHODS USED IN THE STUDY OF PLANT NUTRITION

Enquiries on the subject matter of this book should be sent to the author at Long Ashton, those about the work of the Commonwealth Bureau of Horticulture and Plantation Crops to the Director of the Bureau at East Malling,

Orders and requests for lists of Agricultural Science publications should be sent to The Publishing Manager, Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England.

SAND AND WATER CULTURE METHODS USED IN THE STUDY OF PLANT NUTRITION

by

E. J. Hewitt, B.Sc., Ph.D., A.K.C.
University of Bristol Agricultural and Horticultural Research Station,
Long Ashton, Bristol.

Technical Communication No. 22 of the
Commonwealth Bureau of Horticulture and Plantation Crops,
East Malling, Maidstone, Kent.

COMMONWEALTH AGRICULTURAL BUREAUX

First published in **1952** *by*
Commonwealth Agricultural Bureaux,
Farnham Royal, Bucks, England
and printed in Great Britain by
Bradley & Son, Ltd.
Reading.

CONTENTS

Part One

GENERAL REVIEW

	Page
Objects, scope and definitions	1
Historical	2
Plant containers	4
Drainage	4
Undrained containers	4
Drained containers	5
Capacity	5
Sand culture	6
Water culture	14
Relation to solution renewal and aeration	14
Shape of containers	14
Covers for containers	15
Exclusion of light and heat	15
Exclusion of dust	15
Support of culture plants	16
Composition of covers	17
Composition of containers	17
Metal	17
Cement	18
Wood and paper	19
Plastic	19
Unglazed clay	19
Glazed clay	20
Glass	22
Cleaning containers	23
Multiple compartment containers and "Split root" methods	24
Solid rooting media	26
Particle size of sand	26
Mechanical analysis	26
Choice of particle size	27
Chemical analysis and purity of sand	29
Macronutrients	29
Micronutrients	29
Relation to grading	30
Toxic constituents	30
Sand purification	30
Use of unpurified sand	30
Methods and results using purified sand	30
Mixed solid media	32
Base exchange materials	34
Zeolites and synthetic resins	36
Colloidal clays	38
Other materials	40
Solid nutrient compounds	40
Water	42
Sources of water	42
Rain water	42
Composition	42
Storage	46
Consumption	48
Use	48
Tap water	48
Distilled water	50
Metal stills	50
Non-metal stills	52
Demineralised water	57
Activated carbon	62
Nutrient impurities and purification methods	64
Impurities in nutrient salts and raw materials	64
Phosphates	64
Potassium salts	65
Nitrates	65
Other salts	65
Iron and heavy metal salts	66
Experiments using unpurified salts	66
Tests of nutrient purity	68
Conclusions	69
Methods of purifying nutrient reagents	69
Recrystallisation	70
Distillation methods and extraction of solid reagents	71
Carbon adsorption techniques	72
Phosphate, alkaline earth and related adsorption methods	73
Co-precipitation with organic reagents or sulphides	78
Extraction with immiscible solvents	80
After chelation	80
With ether-hydrochloric acid	81
Other methods of purification	81
Summary of recommended methods	82
Composition of the nutrient solution	83
Terminology	83
Specimen solutions	84
Osmotic pressure, salt concentration and sodium content of nutrient solutions	89

Page

Osmotic pressure 89
Salt tolerance 91
Sodium content 92
Source of nitrogen in nutrient solutions ... 93
Nitrate and ammonium nitrate 93
Relation to pH 93
Solution renewal 94
Season 94
Aeration and micronutrient supply ... 94
Nutrient uptake... 95
Iron requirements 95
Relative tolerance and effect on growth 95
Other sources of nitrogen in culture solutions 97
Influence and control of pH of the nutrient solution 98
Influence of pH 98
Production of specified pH values ... 99
pH stability 100
Control of nutrient pH 101
Supply and utilization of iron in nutrient solutions 102
Solid iron compounds 102
Inorganic salts 103
Organic compounds 105
Other sources 108
Micronutrients supplied in nutrient solutions 108
Micronutrient supplements 108
Micronutrient requirements 110
Threshold and toxicity levels for micro-nutrients 111
Sources of micronutrients 114
Organic supplements for inorganic nutrient solutions 115
Use of radioactive isotopes in culture experiments 116
Methods for the renewal and application of culture solutions 117
Intermittently renewed solutions 117
Leaching 118
Re-use of nutrient solutions 119
Alternating solutions 119
Continuous renewal of solution 119
Methods 120
Mariotte's bottle 120
Constant level cultures 122
Wick cultures 122
Atomised culture solutions 122
Tensiometers and methods based on water loss 122

Page

Distribution of solution 124
Metering pumps and other equipment ... 127
Solution renewal in relation to aeration ... 127
Irrigation techniques specially adapted for sand cultures 129
Aeration of culture solutions and study of root atmosphere 131
Importance of aeration 131
Methods of aeration 133
Sealed cultures for study of root atmosphere 137
Absorption of nutrients from culture media... 138
Tree cultures and large-scale techniques ... 141
Tree cultures 141
Large-scale installations 143
Seed sterilisation, sterile cultures, pest control and the raising of culture plants ... 147
Seed sterilisation 147
Sterile cultures for whole plants 149
Associated growth cultures 150
Pest control measures 150
Methods of germination of seeds and raising of culture plants 151
Comparative aspects of sand and water cultures and choice of technique ... 154
Comparative aspects of sand and water cultures 154
Choice of plants for culture experiments 156
Choice and limitations of technique ... 157
Influence of environment and biological factors on the behaviour of culture plants ... 159
Influence of environment 159
Light 159
Temperature 160
Humidity 160
Season 161
Controlled environment equipment ... 161
Large-scale installations 162
Supplementary light 163
Light integrators 163
Influence of biological factors 163
Influence of the seed on growth 163
Legumes 165
Variability of culture plants 165
Design of experiments 165
Arrangement 166
Design 167
Measurement of responses of culture plants 168
Interpretation of data obtained in culture experiments 169

Part Two

THE LONG ASHTON TECHNIQUE FOR LARGE-SCALE POT SAND CULTURES

	Page
Objects and requirements of technique	171
Basic materials used	172
Containers	172
The clay pot	172
Glass containers	173
Plastic containers	173
Glazed containers	174
Sand	174
Source and grade	174
Treatment before use	174
Routine use of sand purification equipment	178
Effectiveness of purification	180
Water	180
Collection and storage of rain water	180
Consumption in relation to storage capacity	181
Suitability and purification	181
Operation of demineralisation apparatus	182
Reagents for testing water supplies	185
Distilled water	186
Metal stills	186
Glass stills	186
Tap water	187
Nutrient reagents	187
Composition and preparation of nutrient solutions	187
Preparation of nutrient solutions for micronutrient deficiency work	191
Purification of stock solutions from iron, manganese, copper and zinc	191
Removal of copper and zinc	191
Purification of stock solutions from boron	191
Purification of stock solutions from molybdenum	192
Co-precipitation with 8-hydroxyquinoline	193
Co-precipitation with copper sulphide	193
Purification of compounds used to supply iron	194
Removal of manganese, copper and zinc	194
Removal of molybdenum	194
Tentative method for preparation of iron compounds free from manganese, copper, zinc, molybdenum and gallium	194
Purification of manganese compounds	195
Removal of iron	195
Removal of molybdenum	195
Cleaning of glassware	196
Testing nutrient solutions	196
For iron	196
For manganese	196
For copper, zinc, etc.	196
For boron	196
General technique	197
Lay out and treatment code	197
Raising experimental plants	197
Method of planting	198
Treatment of sand at planting time	198
Change of treatment	199
Frequency of watering	199
Pest control	199
Choice of crop	200

	Page
Acknowledgments	201
Bibliography and author index	202
Subject index	233

ILLUSTRATIONS

Fig			*Page*
1		Large-scale still for production of copper and zinc-free water	53
2		Constant level pyrex glass stills	56
3	A, B and C	Pyrex glass constant-level stills used at Long Ashton	58-59
4		Demineralisation of tap water	63
5	A—I	Methods for constant rate delivery of nutrient solution	
	A	(i) Early solution renewal apparatus and (ii) multiple distributor	121
	B	Simple constant level device for water culture	121
	C	Constant-renewal, aeration and constant level devices in water culture	123
	D	Constant rate of renewal device in sand culture	133
	E	Control of solution renewal with automatic change of reservoirs	125
	F	Regulating the flow of liquid into a culture vessel by capillary tube	125
	G	Regulating the flow of liquid into a culture vessel by annular valve	125
	H	Control of solution at high rate of delivery	126
	I	Controlled solution renewal for several water cultures	126
5	J	Multiple aerator and devices for circulation of nutrient solution in water cultures ...	128
5	K	Apparatus for absorption tests from nutrient solutions	130
6	A—D	Automatically irrigated sand cultures	
	A	Automatically operated sand culture using air lift	132
	B	Irrigated sand cultures and aerated water cultures	134
	C	Cross section view of irrigated sand cultures using air pressure	136
	D	Automatic flooding of multiple pot aggregate cultures	138
6	E—G	Tree water cultures and large-scale sand cultures	
	E	Growing apple trees in water cultures	142
	F	Shallow beds for use with automatically operated sand cultures	146
	G	Automatically operated deep sand cultures	148
7	A	Original automatic sand washing equipment	176
7	B	Improved " Keebush " sand digester	177
8		Lay-out and flow sheet for use of ion-exchange water purification equipment ...	181

Plates		*Facing*
A	Biological breakdown of waxed lining in clay pot	20
B	Effect of container on production of iron and manganese deficiencies in oat ...	21
C	Boron deficiency in lucerne and effect of container	22
D	Sand purification equipment at Long Ashton	31
E	Constant-level glass stills at Long Ashton	56
F	Effect of water supply on the production of copper deficiency in tomato in sand culture	57
G	Zinc and copper deficiencies in tomato	179
H	Molybdenum deficiency in lettuce	193
I	Molybdenum deficiency in kale	199

TABLES

		Page
Table 1.	Examples of capacities of containers used in sand culture experiments	7
Table 2.	Examples of capacities of containers used in water culture experiments in relation to provision of aeration, storing and change of solution	10
Table 3.	Relation between size of container, yield of cauliflower, depth of sand and moisture content	14
Table 4.	Comparative data for mechanical analysis of sands used in culture experiments ... ...facing	24
Table 5.	Approximate correspondence between particle size and standard sieve apertures	26
Table 6.	Yields of crops grown in two grades of silica sand	28
Table 7.	Mineral impurities in silica sands ... ... 33 and facing	33
Table 8.	Types and exchange capacity of some base-exchange materials	35
Table 9.	Miscellaneous data on mineral content of rain water, excluding chlorine, nitrogen and sulphur	44
Table 10.	Analyses of rain water obtained at Long Ashton	45
Table 11.	Summary of concentrations of nutrient elements found in rain water at Long Ashton ...	46
Table 12.	Concentrations of zinc causing injury to culture plants	46
Table 13.	Analytical data for tap water samples used in culture experiments	49
Table 14.	Effect of demineralisation on removal of ions from rain water and tap water at Long Ashton	60
Table 15.	Examples of relative efficiency of removal of certain ions from water by demineralisation ...	61
Table 16.	Steinberg's data on effect of nutrient purification on yields of *A. niger* or *Lemna* after omission of various micronutrients	77
Table 17.	Residual concentrations of manganese after treatment by various modifications of the phosphate adsorption technique	78
Table 18.	Examples of molybdenum content in culture materials as determined by microbiological assay with *A. niger* ... ... facing	80
Table 19A.	Composition of nutrient solutions selected to show historical development and typical modern examples ... ... facing	96
Table 19B.	Other typical nutrient solutions classified according to macronutrient salt composition facing	96
Table 20.	Use of magnetite in sand cultures	102
Table 21.	Levels of zinc and manganese in some culture solutions	113
Table 22.	Composition of permanent artificial standards for manganese test	184
Table 23.	Concentration of standard stock solutions	190

FOREWORD

By Sir Edward Salisbury, C.B.E.,
Vice-President and Secretary of the Royal Society,
Director of the Royal Botanic Gardens, Kew.

The methods of sand and water culture originated in pure scientific enquiry more than a century ago and they have proved of the greatest value in the hands of the plant physiologist in assessing nutritional requirements and the role of various ions. These methods have enabled us to establish the fact that plants can be grown successfully, generation after generation, in solutions of pure chemicals and thus dispose of the claim that organic constituents are essential to the nutrition of the green plant, however beneficial these may be for practical culture. The technique has enabled us to study the nutritive requirements of the plant in an effective manner that would have been frankly impossible had the complex medium that we term soil proved essential to efficient culture. This technique that has proved so valuable a tool in the pursuit of fundamental knowledge has also provided a practical means of culture that avoids the sources of infection that a soil may and often does provide.

The discovery of the significance of the micronutrients, however, at once gave added importance to these methods and called for a great improvement of techniques alike with respect to the receptacles employed, the chemicals from which the culture solutions were made and the sand when such is employed.

Dr. Hewitt in this survey has given us a valuable summary of the techniques involved, of their difficulties and how they can be met or avoided. Dr. Hewitt brings to his task the result of his own investigations at Long Ashton in the refinement of methods for the study of deficiencies. He calls attention to his experience that the purest chemicals available commercially are normally contaminated with minute traces of the micronutrients that render them unsuited, without further purification, to the needs of critical experiments where such micronutrients are the object of study. The results of the author's experience in providing remedial methods should be of great value to all who carry out investigations of this character.

Dr. Hewitt has, in fact, provided us here with a compendium of information on this subject. Just because there is much more to be learnt on these topics and a wide field of experimentation yet to be explored this compilation of the state of present-day knowledge is the more valuable and it should find a wide welcome amongst pure scientists, agriculturists and practical horticulturists and indeed all who are concerned in the culture of plants or theoretical aspects concerned therewith.

Part I

OBJECTS, SCOPE AND DEFINITIONS

The preparation of this Technical Communication was undertaken at the request of the Commonwealth Bureau of Horticulture and Plantation Crops on the suggestion of Professor T. Wallace, C.B.E.

The objects of writing it were : firstly to review and assess the numerous sand and water culture methods used in the study of plant nutrition, and secondly to give a detailed description of the method in use to date at Long Ashton for growing plants in large-scale pot sand cultures, together with all necessary information on the materials used, their source and manipulation.

The scope of the first part has been restricted to aspects of the subject connected with research methods ; hydroponic methods and problems mainly concerned with commercial horticultural applications are not considered unless they have a special bearing on the subject of this review.

Certain other points are excluded from this communication, namely : detailed historical treatment beyond that necessary to give some perspective to the development of the methods under review ; soil pot cultures unless having a special bearing on some point ; tissue culture methods except when used for growth (or regeneration) of whole plants ; nutrition of micro-organisms except where the methods are of importance in relation to their application, as, for example, to micronutrient deficiency techniques ; description of results of experiments, except to illustrate the efficiency, applicability or interpretation of a method.

Special attention has been given to methods for study of micronutrient deficiency problems, including the selection of appropriate materials, preparation of pure solutions of nutrient reagents, and sources of contamination.

No special need for definitions arises in this work except as regards the use of the terms major or macronutrient element and micronutrient element. These are convenient for the description of essential or beneficial elements in terms of the amounts normally supplied to, or found in apparently healthy plants. The term macronutrient element is applied here to nitrogen, sulphur, phosphorus, calcium, magnesium, potassium and, where relevant, to sodium, since these elements are usually supplied in amounts exceeding one part per million in solution and often at a hundred times this concentration or more. The corresponding term for nutrients normally required in very small amounts has been the subject of some discussion. The question has been given special consideration by Arnon (1948) (1950 b), who proposed the term *micronutrient element*. This is explicit and quantitative, and avoids the possible wrong emphasis on importance given by the use of the word " minor," or under estimation of the effects of toxic concentrations due to use of the words " trace," " rare," *spuren* or *oligo*-element. The term micronutrient element, briefly referred to as micronutrient, is adopted throughout this work and is applied to the elements manganese, copper, zinc, boron and molybdenum normally required in amounts less than one part per million in solution and often less than one-tenth of this concentration and also to iron, which behaves as a micronutrient and is conveniently treated in this group as regards problems of technique. Elements of indeterminate status in plant nutrition, e.g. aluminium, gallium, silicon, chlorine, cobalt, vanadium and others, are sometimes described by the term potential micronutrient.

HISTORICAL

This historical review is not intended as a reference work on the historical aspects of plant nutrition investigation, but merely as an introduction to the subject as a whole, particularly with regard to the use of culture methods. Further detailed accounts of this aspect may be obtained from the works of Tottingham (1914), Pfeffer (1918), Shive (1940a), Reed (1942), Stiles (1946), and Woodman (1946).

Plant physiology is a relatively recent subject in relation to such other aspects of botany as ecology, taxonomy and anatomy, and dates from the seventeenth century (Reed 1942). Plant nutrition, however, is probably one of the earliest of the many aspects of plant physiology to be studied quantitatively. Van Helmont (1577-1644) made what appears to have been the first quantitative attempt to gain some idea of the origin of the increase of fresh weight of a plant. He grew a weighed willow branch in a weighed amount of soil, the surface of which was protected from dust and supplied only with water, for five years, at the end of which the tree and soil were again weighed. Woodward (1699) made the earliest recorded use of a water culture method without any solid substrate, and grew vetch, potato and mint in water obtained from springs, rivers, conduits, rain and distillation. He concluded that water is merely a carrier of " terrestrial matter." This work was followed 60 years later by experiments by Duhamel du Monceau (1758) who grew oak, almond, chestnut, walnut and beans in cultures with filtered water from the Seine. De Saussure (1804) made the first attempt to use controlled water culture experiments. He grew *Polygonum persicaria* and *Bidens cannabina* in distilled water, and in dilute solutions of various salts. He found that nitrates were necessary in addition to other mineral salts ; that the uptake of solution was not selective against toxic substances ; that the relative amounts of different solutions absorbed varied and that the required amounts of mineral salts can be taken up from a very dilute solution. This work was only extended after an interval of nearly 40 years by Wiegman and Polstorf (see Reed 1942) who concluded that except for the mineral constituents present in the seed, the plant obtained the whole of the rest of its requirements by absorption through the roots. They thus realised that plants can make some development by using the mineral reserves of the seed and were the first to draw attention to the significance of this source of supply. The first elucidation of the essential nature of the macronutrients was attributed by Shive (1940 a) to Sprengel. The importance of iron was recognized by Gris (1844).

Boussingault (*c.* 1851-1856) (cited by Shive 1940 a) was one of the first to use sand culture as a means of study and his work was extended by that of Salm-Horstmar (1849) (1851). The detailed references elsewhere to the latter's experiments are a testimony to his remarkable insight into the problems and scope of culture methods. He grew oats in waxed glass or pure wax containers with sand, quartz or charcoal that had been acid-extracted to remove mineral impurities. He then omitted different elements and produced evidence for the need of nitrogen, phosphorus, sulphur, calcium, potassium, magnesium, silicon, iron and manganese and described visual symptoms of deficiency effects including those of " grey speck " due to lack of manganese. The modern approach to the study of plant nutrition through sand culture methods was thus established at this time. The basis of modern water culture technique rests on the later work of Sachs (1860) carried out at the School of Forestry in Tharandt. Sachs realised that water cultures were in many ways simpler than sand cultures as they avoided the solid phase, and possible associated impurities when search was being made for mineral elements necessary for plant growth. Sachs was the first to use a standardised and relatively simple type of nutrient solution which contained all those elements considered at that time to be essential, namely nitrogen, phosphorus, sulphur, potassium, calcium, magnesium and iron. The solution was prepared from two stock mixtures, one of which contained no phosphates. Manganese sulphate was also included. Sachs also introduced the procedures of changing the solution at intervals, of limiting the plant density according to size and he tried the effects of using ammonium compounds.

The work of Sachs was actually published simultaneously with that of Knop (1860) who paid attention to standardising the methods of producing seedling material by the now widely used method of germination on muslin. Knop (1861) (1865) made further experiments

on variation in composition and introduced further improvements in the nutrient solution, which was simpler than that used by Sachs and based on molar ratios. Knop (1865) published a nutrient solution which was widely used until recently. The importance of changing the nutrient solution was further emphasised by Knop and by Wolf (1866). Mazé (1900) examined the use of ammonium compounds as a source of nitrogen for maize and showed the value of adding calcium carbonate to the cultures.

A systematic, quantitative study with wheat of the effects of nutrient composition, osmotic pressure and concentration was made in classical experiments by Tottingham (1914). In this work a solution which comprised only four principal salts and an iron compound was developed from the study of fresh weight production in eighty-four nutrient combinations and in Knop's standard solution. Tottingham based the nutrient variations on the molar fractions of the four salts that contributed one-tenth increments in total osmotic pressure for three values of osmotic pressure corresponding to 0·05, 2·50, and 8·15 atmospheres. The results were represented with the aid of triangular diagrams introduced to plant physiology by Schreiner and Skinner (1910). The use of molar fractions was not entirely new but was emphasised more than hitherto. Great attention was also paid to the avoidance of precipitation in the culture solution and to establishing the optimum total concentration of salts at about 0·6% or less. This work was extended by Shive (1915 a, b) who further simplified the basal nutrient solution to three principal salts, and this type of " three salt " nutrient solution has been widely used up to the present. The salts used were calcium nitrate, potassium dihydrogen phosphate and magnesium sulphate. Sodium compounds were thus eliminated and an important limitation not explicitly realised then was that the ions—calcium and nitrate, or potassium and phosphate—could not be varied independently. The technique used was similar to that of Tottingham (1914), but the " best " solution, which was superior to Tottingham's " best," had a lower concentration and an osmotic pressure of 1·75 atmospheres. Shive paid considerable attention to methods for raising uniform seedlings and used rotating tables to reduce unevenness in the environmental factors. The development of three-salt type nutrients was completed by Livingstone and Tottingham (1918) who drew attention to the fact that six combinations of the pairs of ions present in the Shive three-salt nutrient were possible.

The development of nutrient solution types was based, as outlined here, on the supply of essential elements in suitable concentrations and ratios, and the use of the simplest formula consistent with satisfactory results. In addition to the classical formula evolved by Shive, other early types of importance and still in general use include those of van der Crone (1904), Hoagland (1919) and the Rothamsted solution. In Crone's solution solid nutrients including ferrous phosphate were added and it is frequently a satisfactory nutrient for avoiding the accidental development of iron deficiency chlorosis prevalent in water culture experiments. Hoagland's (1919) solution was designed to reflect as closely as possible the proportions of nutrient elements in soil solution. The nutrient solution developed more recently by Arnon and Hoagland (1940) was intended to present the nutrient elements in the approximate proportions in which they were found to be absorbed by the (tomato) plant.

The importance of allowing access of air to roots was recognised by Hall *et al.* (1914 a, b) and methods for the continuous renewal of the culture solution probably date from the work of Nobbe (1868) and Schloessing (1899).

The indication that the early type standard nutrient solutions devised by Sachs and others were able to maintain normal growth and development did not prevent the search for other possible nutrient elements required in smaller quantities, and from that time historical events of importance were mainly in connection with developments of this nature and the improvements in technique that were needed for such work. The first micronutrients in addition to iron, for which evidence of essentiality was obtained for fungi or higher plants, were manganese, studied by Salm Horstmar (1851), Raulin (1863), Bertrand and Javillier (1911), (1912 a, b), McHargue (1922) ; zinc shown by Javillier (1907), Mazé (1914), Steinberg (1919), Sommer and Lipman (1926) ; and boron shown by Aghulon (1910), Mazé (1915) (1919), and Warington (1923), who produced convincing proof of the essential nature of this element.

Although Bertrand and Javillier developed special procedures for the purification of nutrient salts and recognised the importance of using appropriate glassware, the most important advance was that made by Steinberg (1919) *et seq.* who introduced an efficient routine procedure for the elimination of certain heavy metals, especially zinc, from culture solutions.

The more difficult proof of the essential nature of copper was achieved independently (and with different degrees of success) by Sommer (1931) and by Lipman and MacKinney (1931). Molybdenum was added to the list for bacteria by Bortels (1930) and for higher plants by Arnon (1938), and Arnon and Stout (1939 b).

Claims have also been made for the essential or beneficial nature of silicon for cereals by Sommer (1926) and beet by Raleigh (1939) and Wagner (1940); of aluminium by Stoklasa (1922) and by Sommer (1926); of chlorine by Lipman (1938) and of gallium by Steinberg (1938 b) (1941) for *Aspergillus* and *Lemna*. These claims need further substantiation and have not greatly stimulated research up to the present. Steinberg (1945 a) reported inability to repeat earlier results after using up a particular batch of sugar in spite of the application of specific methods to remove gallium. Liebig *et al.* (1943 b) also failed to produce a response to gallium with citrus. Evans (1950) noted a beneficial response to cobalt injected into cacao plants. Trelease and Trelease (1938) showed that selenium stimulated growth in *Astragalus racemosus*.

There may, however, yet be other micronutrients essential for plant growth. Arnon and Stout (1939 a) and Arnon (1948) (1950 a) have specified certain criteria of essentiality, to be applied when seeking proof of the essential nature of a given element. Failure to produce a response after attempts to eliminate a particular element from the nutrient solution must be interpreted in terms of those criteria, not to mean that the element is not essential but in a strictly quantitative sense to mean that the element in question, if essential, is required in amounts less than the limit set by the technique employed.

Arnon's criteria are as follows :

1. The plant must be unable to grow normally or complete its life cycle in the absence of the element.
2. The element must be specific and not replaceable by any other.
3. The element must have a direct influence on the plant and be involved in its metabolism.

Potassium satisfies these criteria, as its function cannot be wholly replaced by sodium. Molybdenum satisfies the criteria for higher plants when nitrate nitrogen is given but possibly may not be required by certain organisms in the presence of ammonium nitrogen and its status needs further evaluation in this respect. The ability of vanadium to replace molybdenum in *Azotobacter* but not so far in other plants introduces the concept that one is essential in the absence of the other and that neither is entirely essential if both are present. Other instances of partial exception to the criteria of essentiality may perhaps be anticipated as techniques improve.

PLANT CONTAINERS

The main points to be discussed here are those of size and shape, composition and inertness, and applicability of containers to sand or water culture, and multiple compartment containers adapted for split root experiments. Details of tanks and beds used in large-scale experiments are also given in the section devoted to these methods.

Drainage

Undrained containers for sand cultures. Sand cultures provide natural aeration of the roots more efficiently than water cultures, mainly because drainage of the solution out of the containers assists air to penetrate the pore space vacated by the liquid. Free drainage is now always implied in the operation of sand cultures and stagnation of the culture solution in sand usually leads to unsatisfactory growth unless handled with considerable care and skill. Most containers used are designed to allow the excess fluid to drain from a basal outlet.

Some work, however, has been published where containers were used without free drainage and excess solution was removed by suction. Thus McCall (1916 a, b), McCall and Richards (1918), Shive (1918 a), Wolkoff (1918) used small undrained vessels holding about 1·5 kg. of sand to grow wheat, buckwheat and soya beans. Their cultures were maintained at 15% moisture by removing excess solution (added through a funnel on the surface) through a tube by suction and by weighing the cultures. Johnston (1920) grew potatoes in undrained sand cultures holding 4·5 kg. sand. The solution was added through a central tube leading to the bottom and excess was removed by suction through another tube to produce a constant weight. Hoagland (1919) grew barley in sand cultures in undrained 5-gallon glazed jars. The solution was introduced through a central 1-litre percolator and excess solution was removed by siphoning under reduced pressure. Reed and Haas (1923 a) grew citrus in large tanks or cylindrical cans. In the first experiment excess solution was removed by suction through a tube leading out of the top of the containers, but in the later experiments natural drainage by gravity was substituted. De Haan and Schoorel (1940) and de Haan (1941) grew tea in pots with a constant water table maintained near the bottom of the pot by the level of solution in a reservoir connected to them. Solution was drained off and replaced at intervals.

Drained containers : retention of sand. The great majority of sand culture containers are drained by gravity from a basal outlet. The importance of free drainage has been emphasised by Shive and Robbins (1942). It is necessary to arrange that the sand is retained whilst fluid can escape. A gauze of copper wire was used to retain the sand in clay pots, by Wallace (1921), (1924), (1930), and Davis (1930), for fruit trees, by Beard (1932) for hops, by Hewitt (1944), (1945), (1946 a, b), for several farm and market garden crops, and by Eaton (1931) for large-scale irrigated sand beds. Mitchell (1939) used a monel metal gauze in work with *Pinus strobus*. The use of metal gauze to retain the sand is apparently free from any obvious toxic effects, but is only suitable for experiments with macronutrients and sand not treated with any acid.

Sand is effectively retained by glass wool. This method was used by Blake *et al.* (1937) for apple tree cultures, by Woodman (1939 b) where glass wool was secured by piano wire around a glass tube inserted in the side tubulure and by Meyer (1946). When the drainage exit is a central basal aperture, a watch glass serves to take the weight of the sand off the glass wool. This method was used by Davidson and Shive (1934) for peach tree cultures, by Shive (1938), Hewitt (1947 a, b), (1948 a), Hewitt and Jones (1947), (1949) for vegetables, and by Chapman and Liebig (1938) and by Gauch and Wadleigh (1943) for automatically-operated cultures.

The use of glass wool and a watch glass is particularly useful when acid-treated sand is to be used. For micronutrient deficiency experiments the glass wool and watch glasses can be thoroughly cleaned as described in Part Two. For the most exacting work the watch glass should be placed convex side upwards, but the weight of the sand tends to force the rim of the watch glass against the glass wool and the base of the container and an almost complete seal may occur, especially if the sand is shaken during leaching prior to use. A watch glass only was used by Spencer and Shive (1933), Shive and Robbins (1942), and Robbins (1946).

Eaton (1936) (1941 a) used a porous aluminium silicate tile and gravel to provide free drainage in large sand cultures. Evans (private communication) found that cacao in sand culture was especially sensitive to impeded drainage. It was necessary to accelerate drainage during early growth by a wick leading out of the drainage hole.

Capacity

The capacity of the containers used in sand and water culture experiments varies over a wide range. The choice of size of container is determined partly by cost and ease of manufacture and partly by the fact that changes in nutrient composition occur during growth. These changes are greater for small containers and the degree of precision required in this respect and the problem of eliminating traces of a micronutrient, which become more significant in

a large volume, also influence the size of container most suited to the work. Some plants, e.g. Brassicas and potato, appear to need greater root space. Hoagland and Martin (1923) pointed out that sand and water cultures of the same volume are not comparable. This is because the effective solution capacity of a sand culture may be only 20-30% by volume of the sand. Comparison of equal capacity sand and water cultures is also invalidated because of the difference in freedom of diffusion of nutrient ions and the relative number of collisions between them and root absorbing surfaces; the critical levels of nutrient ions were found by Chapman and Liebig (1940) to differ widely between sand and water cultures.

A summary of examples of container sizes selected in sand and water culture experiments is presented in Tables 1 and 2, where details of crop density, frequency of renewal and inclusion of aeration or stirring are given.

Sand culture. Probably the smallest sand culture vessels on record were those used by Salm-Horstmar (1851). They were made of paraffin wax and measured 6 cm. by 3 cm. and held only 60 g. of sand. Colwell (1943) used 1 lb. cannery tins for standardising the sunflower biological boron test. The capacity of pots used in early work was probably insufficient. The containers used by McCall (1916 a, b), Shive (1918 a) and Wolkoff (1918) for cereals and buckwheat held only 1·5-2·5 kilo and Johnston (1920) attempted to grow potatoes in only 4·5 kilo sand. Wallace (1922) used 6-inch pots holding 2·5 kilo for strawberry plants and 10-inch pots for apple trees. Later 18-20 inch diameter pots were recommended for tree fruits by Wallace (1924) (1930). This size of container is satisfactory for the study of deficiency effects in trees on dwarfing or semi-dwarfing stocks such as Malling IX or II and several experiments have been made on tree fruits grown in 2-3 gallon containers holding 14-20 kilo sand, both with intermittent application and continuous flow of nutrient solution as shown in Table 1. Extensive experiments with vegetables have been carried out by the writer with 10-inch pots. Recent experiments at Long Ashton by E. J. Winter (1949 unpublished)* have shown that the " economic " pot size for a plant such as cauliflower grown with a complete nutrient solution is probably the 12-14 inch size when the nutrient is applied intermittently. Pots smaller than the 10-inch size caused considerable reduction in growth and delayed flowering, whilst the largest (20-inch pots) resulted in wilting during early growth owing to the inadequate moisture in the upper regions of the sand. This is due to the fact that the moisture content of the freely drained sand depends on the depth of sand and its particle size. The data given in Table 3 show that the upper regions of the sand in a large container may be near the wilting point (note especially the values marked (S)) and confirm the observations of Lebedeff (1927). Davidson (1946) drew attention to the importance of this relationship in selection of the size of container and particle size of sand (discussed in the Section on rooting media) and emphasised that deep containers need a finer grade of sand than shallow ones. It was found by Winter,* when the largest pots were emptied that a " pan " or concretion had formed at about the depth (6 inches down) where capillary moisture began to increase. This feature appears to be novel in sand cultures.

It would appear that for sand cultures with intermittent application of nutrients, containers holding not less than 10-14 kilo sand are suitable for many vegetables and those holding 40-100 kilo are satisfactory for single tree and bush fruit cultures. Larger containers (Table 1) were used for holding several plants by Arnon and Hoagland (1940), and in a few experiments on fruit trees containers holding up to 22½ tons of sand were used by Lagasse (1929). It must, however, be remembered always that, where purified sand is to be used to eliminate minute traces of micronutrients, large containers may be a disadvantage. Small cultures holding as little as 0·7 kilo may find special application in short-term experiments involving numerous replications, as for example in the work of Ferres and Trumble (1943), on the availability of nutrients in soils as determined by pot tests with small plants. Cook and Millar (1946) showed that growth of beet in soil cultures was directly related to pot size, but not appreciably affected by plant density.

---

* These experiments were made by Mr. E. J. Winter of the National Vegetable Research Station whilst at Long Ashton and use of the data is gratefully acknowledged.

TABLE 1

Examples of Capacities of Containers Used in Sand Culture Experiments.

Reference	Size of Container	Approx. wt. of sand	Plants grown	Remarks
I. *Intermittent Application with Undrained Containers.* (Rate of application daily or less frequently)				
Salm-Horstmar (1851)	6 × 3 cms.	60 g.	Oat 1 per pot	Probably smallest sand cultures on record.
McCall (1916 a, b)	—	1·5 kilo	Wheat 5 per pot	Careful control of watering essential.
Wolkoff (1918)	—	1·5 kilo	Soya bean 3 per pot	,, ,, ,, ,, ,,
Shive (1918 a) (1920 b)	1400 ml.	2·5 kilo	Buckwheat and wheat	,, ,, ,, ,, ,,
Johnston (1920)	—	4·5 kilo	Potato 3 per pot	Probably inadequate rooting space.
Hoagland (1919)	5-gallon	30 kilo	Barley 5 per pot	Adequate root space.
De Haan and Schoorel (1940), de Haan (1941)	27L (6-gal.) cubic glazed pot	42 kilo	Tea 5 per pot	Potassium and other deficiencies.
II. *Freely Drained Containers. Intermittent Application*				
Colwell (1943)	1 lb. cannery tins	0·5 kilo	Sunflower 1 per pot	Biological assay of available boron.
Wallace (1922)	6″ clay pot	2·5 kilo	Strawberry 1 per pot	Deficiency treatments.
Davis *et al.* (1934)	,,	2·5 kilo	,, ,,	,, ,,
Woodman (1940 a, b)	,,	6 kilo	Onion or lettuce 1 per pot	Optimum nutrient levels.
Hewitt and Jones (1947) (1949) (1950) (1951)	5L pyrex beaker or 1-gallon pyrex urn liner	7·5 kilo	*Brassicae* 1 per pot Clovers 5 per pot Cereals 10 per pot	Molybdenum, copper and zinc deficiencies.
Wallace (1922)	10″ clay pot	9·5 kilo	Apple trees (Cox's orange pippin) on broad-leaved Paradise stock	Deficiency treatments.
Hewitt (1944 onwards)	10″ clay pot	9·5 kilo	Wide range of crops: e.g., 10-15 cereal; 3-5 legume; 1-4 beet; 1 tomato; 1 potato or 1 *Brassica* per pot	Deficiency and toxicity treatments and nutrient interrelationships.
Tyner (1935)	2-gallon glazed pot	10 kilo	Maize 3, clovers 20, other cereals, pea, soya bean 10 per pot	Availability of felspar.
Gregory and Crowther (1928)	2-gallon glazed pot	14 kilo	Barley 3 per pot	Solid nutrient reagents used in deficiency experiments.
Gregory and Richards (1929)	2 ,, ,, ,,	14 kilo	,, ,,	,, ,, ,,

[*continued overleaf*

TABLE 1 (*contd.*)

Reference	Size of Container	Approx. wt. of sand	Plants grown	Remarks.
Lineberry and Burkhart (1943)	2-gallon glazed pot	14 kilo	Strawberry 2 per pot	Deficiency experiments.
Davis (1930)	12″ clay pot	14 kilo	Fruit trees 1 per pot	,, ,,
Wallace (1930)	12″ ,, ,,	14 kilo	,, ,, ,,	,, ,,
Hill and Johnston (1940)	12″ ,, ,,	14 kilo	,, ,, ,,	,, ,,
Blake *et al.* (1937)	3-gallon glazed pot	21 kilo	Apple or peach trees 1 per pot	,, ,,
Cullinan *et al.* (1938)	3 ,, ,, ,,	21 kilo	,, ,, ,,	,, ,,
Waltman (1940)	3 ,, ,, ,,	21 kilo	,, ,, ,,	,, ,,
Batjer and Degman (1940)	3 ,, ,, ,,	21 kilo	,, ,, ,,	,, ,,
Woodman (1939 a, b) (1941 b)	3-gallon glazed pot	22 kilo	Lettuce or cabbage 1 per pot	Optimum nutrient levels.
Brown (1945)	4-gallon bins	28 kilo (approx.)	Peach trees 1 per bin	Absorption experiments.
Loustalot *et al.* (1950)	4-gallon jars	,, ,,	Tung seedlings 1-3 per jar	Relation of nutrition to growth, etc.
Roy and Gardner (1946)	8-gallon glazed pot	55 kilo (approx.)	Orange tree 1 per pot	Absorption experiments.
Davis (1930)	18″ clay pot	70 kilo (approx.)	Apple tree 1 per pot	Deficiency treatments.
Wallace (1930)	18″ clay pot	70 kilo (approx.)	Apple tree 1 per pot	,, ,,
Beard (1932)	18″ clay pot	70 kilo (approx.)	Hop 1 per pot	,, ,,
Reed and Haas (1923 a)	Bins 20″ diam. × 26″ deep	200 kilo (approx.)	Citrus tree 1 per pot	Absorption experiments.
Haas (1949)	,, ,, ,, ,,	200 kilo (approx.)	Citrus trees 3 per pot	Potassium nutrition.
Alben *et al.* (1942)	Cylindrical drums, 55 gal.	350 kilo (approx.)	Pecan tree 1 per pot	Deficiency treatments.
Arnon and Grossenbacher (1947)	460 L. (105-gal.) tanks	630 kilo	Tomato 20 per tank	Use of ion exchange materials.
Arnon and Hoagland (1940)	50 cu. feet tanks (312·5 gal.)	2,000 kilo (approx.)	Tomato 20 per tank	Absorption experiments.
Hayward *et al.* (1946)	300 cu. ft. concrete tanks	12 tons (13,000 kilo approx.)	Peach tree 1 per tank	Salt tolerance.
Lagasse (1929)	12 ft. diam. concrete pits	22·5 tons (24,000 kilo)	Peach tree 1 per tank	Nutrient treatments.

111. *Constant Renewal (Drip Cultures) or Frequent Irrigation*

Arnon and Grossenbacher (1947)	5″ clay pots	2 kilo (approx.)	Tomato 3 per pot	Frequent irrigation. Absorption experiments.
Behrens (1928)	5½ ″	1·4 kilo	Oat 5 per pot	700 ml./day Minimum nutrient level.
Tyner (1935)	6″ ″	2·5 kilo	Buckwheat or clover 8 per pot	2 L/day. Minimum nutrient level.
Shive (1938)	—	—	Tobacco, radish, lettuce, cotton	1-2 L/day. Boron deficiency.
Addoms (1937)	3-L. jars	4·5 kilo	Loblolly pine 1 per pot	18 L/day. Growth responses in culture.
Eaton (1941 a)	6·5-L. jar	5 kilo sand + 1 kilo gravel	Tomato 1-2 per pot	Frequent irrigation. Miscellaneous.
Pirschle (1929) (1931 a)	6-L. (approx.) jar	6 kilo	Cereals, *Brassicae*, legumes	5-8 L/day. Effects of nutrient composition.
Shive and Robbins (1942)	5-7·5 L. jar	6-9 kilo (approx.)	Tomato and similar plants 1 per pot	2 L/day. Miscellaneous.
Robbins (1946)	5-7·5 L. jar	6-9 kilo (approx.)	″ ″ ″	
Davidson (1928)	2-gallon jars	14 kilo (approx.)	Peach 1 per pot	1-15 L/day. Effect of nutrient composition.
Davidson and Shive 1934)	2-gallon jars	14 kilo (approx.)	Peach 1 per pot	2-8 L/day. Effects of pH.
Wadleigh *et al.* (1937)	2-gallon jars	14 kilo (approx.)	Maize 5 per pot	Iron nutrition.
Chapman and Liebig (1938) (1940)	2-gallon (9-L.) jars	14 kilo (approx.)	Tomato or citrus	Frequent irrigation. Effects of nutrient concentration.
Clark (1941)	12″ pot	14 kilo (approx.)	Strawberry 5 per pot	12 L/day. Effect of pH and nitrogen supply.
Nightingale (1934)	12-L. (2·5 gall. approx.)	18 kilo	Apple 3 per pot	36 L/day. Effect of nitrogen supply.
Clark (1933)	3-gallon glazed pot	20 kilo (approx.)	Strawberry 4 per pot	12 L/day. Effect of pH.
Spencer and Shive (1933)	3-gallon glazed pot	20 kilo (approx.)	Rhododendron 2 per pot	1 L/day. Growth in sand culture.
Guest and Chapman (1944)	3-gallon jars	20 kilo	Orange seedlings 3 per pot	Frequent irrigation. Effects of pH.
Gauch and Wadleigh (1943)	5-gallon pots	30 kilo	Farm, market garden and soft fruit crops	Frequent irrigation. Miscellaneous.
Chapman and Liebig (1938)	25-gallon	150 kilo	Orange 1 per pot	Frequent irrigation. Growth experiments.
Eaton (1931) (1942 a) (1944)	18 cu. ft. beds	700 kilo (approx.)	Vegetable and fruit crops several per bed	Frequent irrigation. Boron deficiency and excess and salt tolerance.
Eaton (1936)	40 cu. ft. beds	1,600 kilo (approx.)	″ ″ ″	Frequent irrigation. Miscellaneous.
Thomas *et al.* (1943 a)	90 cu. ft.	4,300 kilo (approx.)	Several farm crops	Frequent irrigation. Controlled environment.
Eaton (1936)	180 cu. ft.	7,000 kilo (approx.)	Fruit trees	Frequent irrigation. Salt tolerance.
Eaton (1941 a)	300 cu. ft.	13,000 kilo	Fruit trees	″ ″ ″ ″
Hayward *et al.* (1946)	300 cu. ft.	13,000 kilo (12 tons)	4-year-old peach trees 2 per bed	Irrigation 4 times daily. Growth experiments.

TABLE 2

Examples of Capacities of Containers Used in Water Culture Experiments in Relation to Provision of Aeration, Storing and Change of Solution

I. *Solutions Renewed Intermittently*

Reference	Volume of Container	Plants grown	Provision of Aeration and Stirring*	Period of renewal	Type of Experiment
Duggar (1920 b)	240 ml. glass jars	Wheat or maize	—	Not renewed	Use of solid (suspended) nutrients
Schreiner and Skinner (1910)	250 ml. ,,	Wheat 10 per jar	—	3 days	Absorption experiments
Shive (1915 a, b)	250 ml. ,,	Wheat 6 ,,	—	3 ,,	Nutrient composition experiments
McLean and Gilbert (1926)	250 ml. ,,	Rye 6 ,,	—	Alternating soln. 3½ days	Toxicity tests (aluminium)
		Cabbage or rye 1 per jar	—		
Tottingham (1914)	250 and 400 ml. jars	Wheat 6 per jar	—	3½ and 7 days	Nutrient composition experiments
Löhnis (1937)	300 ml. jars	Several species 1 per jar	—	14 days	Boron deficiency
Brenchley (private communication 1949)	½- and 1-pint mls.	275-550 Several species 1-3 per jar according to size	—	7-14 days	Boron deficiency and other experiments
Shive and Martin (1918 a, b)	515 ml. glass jars	Buckwheat 3 per jar	—	4-5 days	Nutrient requirements
Warington (1923)	600 ml. ,,	Broad bean and others 1 per jar	—	7 days	Boron deficiency
Warington (1946)	400-800 ml. ,,	Lettuce 1 per jar	—	14 days	Molybdenum deficiency
Greenwood (1951)	800 ml.	Cacao 1 per jar	—	4 weeks	Micronutrient experiments
Barnette (1921)	250, 500 ml. 1-L., 2-L. jars	Wheat 1 per jar	—	3½ days	Relation of container size to growth
Reed and Haas (1924 a)	1-L., 2-L. jars	Citrus 3 per jar	—	7 days	Effect of nutrient composition
Sommer (1931)	1-L. pyrex beaker	Tomato, flax, sunflower	—	—	Copper deficiency
Smith and Bayliss (1942)	1-L. ,, ,,	*Pinus radiata* 5 per beaker	A	—	Zinc deficiency
Stiles (1915)	1-quart (1·2-L.) jars	Wheat or barley 1 per jar	—	30-40 days	Nutrient concentration experiments
Tarr and Noble (1922)	1-quart (1·2-L.) jars	Wheat 5; soya bean 3; or maize 1 per jar	—	—	Effects of pH
Franco and Loomis (1947)	1-quart (1·2-L.) jars	Maize, *Brassicae*, soya bean, tomato, 2 per jar	—	3½ or 7 days	Absorption experiments
Mulder (1948 a)	1-quart (1·2-L.) jars	Tomato, pea, barley, 2 per jar	—	—	Molybdenum deficiency

s'Jacob (1939)	350 ml.-1·5-L. jars	... Coffee 1 per jar, capacity increased with age	—	—	Nitrogen concentration tests
Sachs (1860)	1·5-L. jars ...	... Maize 5 per jar	—	8-12 weeks ...	Early nutrition experiments
Sideris *et al.* (1938)	1·8-L. in 2-L. jars	... Pineapple " slip " 1 per jar	—	7 days ...	Effects of source of nitrogen
Lipman and MacKinney (1931)	2-L. pyrex beakers	... Barley	—	6½ weeks ...	Copper deficiency
Stout and Arnon (1939) ...	2-L. ,, ,,	... Tomato plants 8 per beaker	—	3 weeks ...	Micronutrient deficiencies
Millikan (1942) (1944) ...	2-L. ,, ,,	... Wheat *and* flax 4 each per beaker	—	3-4 weeks ...	Zinc and copper deficiencies
Smith (1943)	2-L. ,, ,,	... *Pinus radiata*	A	—	Micronutrient deficiencies
Wade (1944)	2-L. ,, ,,	... Citrus seedlings 2 per beaker	A	6-8 weeks ...	Copper deficiency
Breazeale (1923)	2·5-L. jars ...	... Wheat 500 per jar ...	—	Daily ...	Absorption experiments
Lehr (1940)	2·5-L. ,, ...	... Mustard 5-12 per jar ...	A	6-10 times during growth	Boron deficiency
Samuel and Piper (1929) and Piper (1941) (1942) and (private communication)	3·2-L. ,, ...	... Cereals, legumes, tomato 6 per jar	— A (later experiments)	Twice during growth	Manganese and copper deficiencies
Wolf (1866)	3·5-L. jars ...	... Cereals and legumes ...	—	7-10 days ...	Early water culture work
Hoagland *et al.* (1935) ...	4-L. pyrex beakers	... Citrus and stone fruit seedlings	—	—	Zinc deficiency
Hoagland (1941)	4-L. ,, ,,	... Plum seedlings 1 per jar	A	—	Molybdenum and copper deficiencies
Mazé (1915)	4-5-L. jars ...	... Maize 1 per jar	—	—	Boron deficiency
Tanada (1946)	4-5-L. (1-gall.) jars	... Coffee 1 per jar	A	21 days ...	Relation of light to nitrogen uptake
Zinzadze (1926)	5-L. jars ...	... Maize 2 per jar	A	55 days ...	Effects of nutrient composition
Birner and Lucanus (1866) ...	5-6-L. jars ...	... Oat 5 per jar	—	55 days ...	Early culture experiments
Parker (1927)	7·5-L. jars ...	... Maize 1 or 2 per jar ...	—	Main solution renewed weekly PO_4 level twice daily	Absorption experiments
Bartholomew and Janssen (1929)	7·5-L. jars ...	... Cereals, legumes, cotton 2-6 per jar	—	K level renewed twice daily	Absorption experiments
Liebig *et al.* (1942)	7·5-L. pyrex vessels	... Citrus seedlings	A	Frequent alternation between solns.	Copper toxicity
Liebig *et al.* (1943 b)	8-L. pyrex vessels	... Citrus seedlings 3 per jar	A	28 days ...	Effects of gallium deficiency

[*continued overleaf*

TABLE 2 (*contd.*)

Reference	Volume of Container	Plants grown	Provision of Aeration and Stirring*	Period of Renewal	Type of Experiment
Hoagland *et al.* (1935) ...	10-L. earthenware jars	Apricot seedlings... ...	A	7 days ...	Zinc deficiency
Clements (1928)	12-L. asphalt-painted glazed jars	Pea 40 per jar	—	7 days ...	Interrelations of nutrient and length of day
Ramig and Vandecavye (1950)	3-gall. (14-L.) glazed jars	Raspberry 1 per jar ...	A	21 days ...	Nutrient requirements
Eaton (1941 a)	14·5-L. glazed vats ...	Several plants	A	—	Miscellaneous
Smith (1943)	15-L. bitumen-painted iron tanks	*Pinus radiata*	—	—	Boron deficiency
Colby (1933)	18-L. bitumen-painted jars only ½ full	French prune 1 per jar ...	A	3-4 weeks ...	Absorption experiments
Schappelle (1942)	18-L. pyrex jars ...	Pineapple 1 per jar ...	A	4 weeks ...	Effects of pH and nutrient composition on growth
Sideris *et al.* (1943)	18-L. ,, ,, ...	Pineapple 1 per jar ...	A	2 weeks ...	Effects of iron supply on growth
Pearse (1937) (1940)	18-L. jars	Apple trees 1 per jar ...	A	2-3 weeks ...	Growth experiments
Haas (1937)	22·5-L. *shallow* pans ...	Citrus seedlings	—	—	Nitrogen nutrition
Sideris and Young (1946) ...	5-gall. (22·5-L.) crocks	Pineapple "slips" 16 per jar	A	2 weeks ...	Relationship of nitrogen and growth
Arnon *et al.* (1942)	40-L. tanks	Tomato 6, lettuce 10 or Bermuda grass 11 per tank	A S	—	Absorption experiments
Hoagland and Snyder (1933)	45-L. bitumened tanks	Strawberry 10 per tank ...	—	Twice during experiment	Boron deficiency and salt tolerance
Hoagland *et al.* (1935) ...	45-L. bitumen-painted iron tanks	Tobacco, maize, apricot, etc., alfalfa, squash, 12 per tank	A	—	Zinc deficiency
Houghland (1947)	95-L. vats	Potato 1 per vat ...	A	PO_4 level renewed twice daily	Absorption experiments
Parker and Pierre (1928) ...	100-L. bitumen-painted cans	Maize 3 per can	A	Renewed twice daily	Absorption experiments and minimum levels
Guest and Chapman (1944) ...	100-L. vats	Citrus seedlings 6 per vat	A	Renewed according to Na level	Effects of pH
Arnon (1937)	115-L. bitumen-painted iron tanks	Barley 64 per tank ...	A	Weekly ...	Absorption experiments

Arnon and Johnson (1942)	115-L. bitumen-painted iron tanks	Tomato, lettuce or Bermuda grass 6-10 per tank	A S	Weekly	Absorption and pH experiments
Houghland (1950)	30-gall. glazed tank	Potato 1 per tank	A	—	General
Arnon and Hoagland (1940) and Arnon and Grossenbacher (1947)	460-L. bitumen-painted iron tank	Tomato 20 per tank	A	Frequently	Growth and absorption
Chapman and Parker (1942)	600-L. glazed conduit pipe	Citrus 1 per tank	A	4 weeks (extra N weekly)	Absorption tests
Chapman *et al.* (1947)	700-L. asphalt-lined tanks	Citrus, mature trees 1 per tank	A	Monthly. K level restored weekly	Minimum and maximum K levels
Tidmore (1930)	1,000-L. tanks	Maize, tomato and sorghum 3 each per tank	A	PO_4 level renewed 3 times daily	Absorption and minimum level experiments
Sommer (1936)	1,000-L. asphalt-lined tanks	Wheat, pea, tomato, cotton, maize and buckwheat, 8 each per tank	A	PO_4 level renewed daily	Absorption and minimum level experiments

II. *Solution replaced by Continuous Renewal Method*

Stahl and Shive (1933)	1-quart (1·2-L.) jars	Oat, buckwheat, 3 per jar	—	1 L. per day	Absorption and growth experiment
Jones and Skinner (1927)	1-quart (1·2-L.) jars	Maize or soya bean 1-3 per jar	A	1 L. per day	Absorption and growth experiment
Zinzadze (1932)	Not specified	Maize 3 per jar	A	Not specified	Nutrient pH and composition
Shive and Robbins (1942)	1-, 2- or 8-quart (1·2-L.) jars	Maize 3 or tomato 1 per jar	—	1-2 L. per day	Miscellaneous experiments
Pirschle (1929) (1931 a)	3-L. jars	Cereals, *Brassicae*, legumes, etc.	—	8 L. per day	Effect of nutrient composition
Marsh and Shive (1925)	6-L. jars	Soya bean 3 per jar	—	1 L. per day	Supply of iron in solution
Teakle (1929)	16-L. jars	Wheat 10-20 per jar	—	1-8 L. per day per plant	Absorption experiments
Johnston and Hoagland (1929)	5-gall. (22·5-L.) jar	Tomato 5 per jar	—	1·2-8 ml/min./plant = 1·8-11·5 L. per day	Effect of nutrient concentration
Clark and Shive (1934)	5-gall. (22·5-L.) jar	Tomato 3 per jar	A	1·4 L. per day	Effect of pH on absorption

* A = Aerated. S = Stirred.

Water culture. Water culture containers also show considerable range of size (Table 2). Early work of Schreiner and Skinner (1910), Tottingham (1914), Shive (1915 a, b), (1918 a, b), Duggar (1920 b) was often carried out in containers that were probably too small in relation to the number of plants and frequency of solution renewal. A popular and useful size is the 2 L. beaker, extensively used for micronutrient deficiency studies. It is interesting to note that Birner and Lucanus (1866) allowed 4-5 litres for 5 oat plants whilst Breazeale (1923) grew up to 500 wheat seedlings in a single 2·5 L. container. Barnette (1921) grew *single* wheat plants in containers of ·25, ·5, 1 and 2 L. capacity. The solution was changed every $3\frac{1}{2}$ days, but there was a progressive increase in yield up to the largest capacity and chlorosis was also longest delayed. It will be seen from Table 2 that the largest water culture containers of about 1,000 L. are smaller than those recorded in sand culture experiments even when equal volumes of solution are compared. Where larger containers were used the plant density per litre was usually considerably less than in small containers.

TABLE 3. (Data of E. J. Winter)

Relation between Size of Container, Yield of Cauliflower, Depth of Sand and Moisture Content

Size of Pot	% Moisture remaining at depths indicated after saturation and drainage for 5 hours					Weight of sand kilo	Yield g. (fresh wt.)
	Depth† 1	2	3	4	5		
5″	23	13 (S)	—	—	—	1	308·3
6″	—	—	—	—	—	2	683·8
10″	21	15	7 (S)	—	—	10	1751·3
12″-14″	20	8	—	3 (S)	—	20	2650·0
20″	18	5	—	2	2 (S)	100	3652·5

†1. At drainage hole. 2. 5 inches above. 3. 8·5 inches above. 4. 10 inches above. 5. 15 inches above. (S) Surface level. Sand grading 85% 0·5-1·0 m.m.

Relation to solution renewal and aeration. In general, smaller containers are more suitable for both sand or water culture work when continuous solution renewal or frequent intermittent irrigation is arranged, than when the application or change of solution is relatively infrequent, but frequent irrigation of deep sand beds is also needed to avoid wilting of small or shallow rooting plants owing to the drainage effect already mentioned. It cannot be assumed, however, that continuous renewal of nutrient solution justifies the use of an unduly small container. Eaton (1936) expressed a preference for large-volume stirred cultures as opposed to small-volume constant solution renewal methods, where water culture methods were used.

The question of aeration is considered in a separate section, but examination of Table 2 shows that aeration is usually deemed to be a necessary feature of water culture technique when the capacity of the container exceeds 3 L., and there can be little doubt that aeration is beneficial in even smaller cultures. Stirring is occasionally employed for special purposes as in critical tests of nutrient absorption or pH effects, carried out by Arnon *et al.* (1942) and Arnon and Johnson (1942). Usually, however, aeration causes sufficient disturbance to make additional stirring unnecessary as concluded by Parker and Pierre (1928), Hoagland and Broyer (1936) and Arnon (1937). Aeration is less frequently provided in constant renewal types of water culture than in the static types as shown in Table 2.

Shape of Containers

Little work seems to have been published on the importance of the shape of the container. According to Hubbell (1930) the standard clay pot and the glazed crock of similar capacity were equally suitable for *Pelargonium hortorum* grown in soil but a number of others were inferior, especially where the surface area to volume ratio was small, but Lehr (private communication) recommended deep slender pots for sugar beet to accommodate the tap root. As already mentioned the depth of container is related to texture of sand.

For water culture work the surface area may be important. Wide, shallow vessels give better natural aeration and should be used if forced aeration is not employed as suggested by Haas (1932 b), (1937), Hoagland and Broyer (1936), Hoagland and Arnon (1939). They should not exceed 8-9 inches depth and there should be at least 2 inches between the loose fitting cover and the surface of the solution to allow of maximum aeration by diffusion. Haas (1932 a, b) also pointed out that shallow containers need care in management as drying out of the roots may easily occur. Zinzadze (1932) favoured vessels with a conical section when aeration was used with static nutrient supply, as he considered this shape promoted maximum circulation in the nutrient medium.

Covers for Containers and Plant Supports

Covers are not always convenient or necessary, but where used they serve a number of purposes. These include the provision of shade and reduction of water loss, the exclusion of much of the dust in the air and the most practical means of support in water cultures.

Exclusion of Light and Heat from Glass Containers

When glass containers are used for water or sand culture it is necessary to exclude light. This is effectively done by using two or more layers of thick brown paper, as recommended, for example, by Hoagland and Arnon (1939), and Brenchley (private communication). Millikan (1942) (1944) used gold size in conjunction with black paint. The use of paint has the disadvantage that the coating may cause contamination or inconvenience during the rigorous acid or steaming treatments used for cleaning containers and, once applied, also prevents inspection of the culture through the walls. Two layers of material, the first black to exclude light and the outer one white to reflect heat have also been used. Thus black and white paper was used by Zinzadze (1932) and Löhnis (1937) ; black and white or aluminium paints were used by van Schreven (1934 b), Chapman *et al.* (1937), Stout and Arnon (1939), Vanselow and Datta (1949). Incidentally, Davidson (1944) used aluminium paint on the exterior of clay pots to increase reflection and to reduce heat absorption. Outer covers or boxes also serve to reduce temperature rise. Wallace (1924), (1928), (1930), Beard (1932), Read (private communication) used wood boxes to shelter sand cultures for fruit trees. Lehr (1940) used boxes to shelter water cultures (groups of 10) used for boron deficiency tests. Evans *et al.* (1950) placed glass beakers used to contain sand inside glazed earthenware jars.

Exclusion of Dust from Cultures

The exclusion of dust may be of great importance in some circumstances. Stout *et al.* (1947) recorded that the zinc contamination present in the dust that accumulated on the covers of water cultures was more than that normally included in the nutrient solution and was able, on addition to the zinc deficient cultures, to restore normal growth. Hewitt (unpublished) exposed clean 3 × 1-inch glass slides horizontally for 1 month in a greenhouse used for culture experiments. The following accumulations of micronutrients were recorded :—

Element	Fe	Mn	Cu	Zn	B	Mo
Accumulation as mg/sq. foot/month	0·1	0·02	0·03	0·02	0·06	less than 0·003

Smith and Bayliss (1942) used a greenhouse treated with zinc-free paint for zinc-deficiency studies. Evans *et al.* (1950) avoided introduction of dust into molybdenum-deficiency sand cultures by adding solution through a perforated flask resting on the surface of the " sand ", which was covered, and protected the top of the flask with a small beaker.

Covers are less easily provided for sand than for water cultures. The reason for this is more clearly understood when cereals, raspberries or other plants with a tillering habit are considered. In water culture the plants are held just below the tillering level and some of the roots are exposed to moist air below the cover. In sand culture, tillers arise at sand level, and this fact makes the provision of covers impracticable. Similar difficulties apply to the provision of covers for root crops grown in sand culture, or where numerous individuals are

grown in a single container. Owing to continued growth of aerial parts in relation to a relatively immovable root system the distribution and size of holes in the cover also cause difficulties. The use of elastic sheet materials, with or without glass tubular guides might be feasible for certain crops like cereals but does not appear to have been recorded. For erect, single-stemmed plants such as tomato, many Brassicae and fruit trees, covers of card, wood, plastic board, tarpaulin or metal in two halves or with a central hole and a slit were used by Wallace (1924) (1925) (1930), Eaton (1936), Waltman (1940), and Johnston and Hoagland (1929).

Support of Culture Plants

In sand culture, as in soil, plants are mainly supported by their root development. Additional support is obtained by attaching a wire frame around the plants or by the insertion of a glass rod into the sand to serve as a stake, or by a wood or metal rod attached to the pot as shown by McHargue (1923 a) and Robbins (1946).

Where plants are not moved about, a useful method is to trail twine from the greenhouse cross-bars or wiring, or to run two or more parallel horizontal lines of strong twine between stakes at intervals and to tie the plants to these where convenient. This method avoids the need to insert anything into the sand or to cause the foliage to come into contact with wire (metal). The use of glass rods, although free from contamination, is not always satisfactory as such rods may easily snap. Glass tube of the same weight has greater strength and if sealed at the lower end might well be reinforced inside by a wooden dowel rod of suitable diameter.

In water culture the cover is the primary means of support. Allowance has to be made for the increase of girth of the plant during growth. If another or larger container is to be used later in the work, the cork or cover must either fit the new container or else removal of the plants must be possible without injury. This is easily arranged, if the stem is held in a hole in a cork with a slot extending from the side, as was used by Sachs (1887) (cited by Hoagland and Arnon (1939)), or if the plants are held by corks inserted in holes in a larger lid ; Parker (1927), Hoagland and Snyder (1933), Hoagland *et al.* (1935), Hoagland and Broyer (1936), Hoagland and Arnon (1939), Arnon and Johnston (1942), Arnon *et al.* (1942).

Hoagland (1919) supported young seedlings in the hole in a cork by means of a wedge of cork cut so that greater space was allowed to the expanding stem by raising the wedge. A central wedge made from a conical cork was used by Tottingham (1914), Tarr and Noble (1922) and others to hold up to six plants in a larger cork stopper, arranged so that the plants were held in a ring of six holes each intersected by the larger central hole meant to take the circular wedge.

Fruit trees in water cultures were supported by loosely fitted split corks by Colby (1933), Pearse (1937), (1940). Painted or varnished wood plugs were used by Chapman *et al.* (1937) and Liebig *et al.* (1943 b). Ramig and Vandecavye (1950) used cotton wool to support the raspberry canes in water cultures.

Cotton wool packing and split corks are used at Rothamsted, Brenchley (private communication), to support most herbaceous plants until large enough to be supported by the cork alone, when the cotton is removed. Cotton "batting" was also used by Tarr and Noble (1922). Cotton wool plugs were also used by Löhnis (1937), Stout and Arnon (1939), Shive and Robbins (1942) and Asana (1945) to support herbaceous plants in small holes in the covers. The cotton wool should be the *non-absorbent* type. Paraffined cotton wool was used by Piper (1942) for manganese deficiency trials. Glass wool or sponge rubber were used by Lehr (1940) for boron deficiency experiments. Millikan (private communication) used small squares of waxed paper sealed by wax to the covers to support plants like flax and to exclude dust.

Dunn (1939) used a pocket of sand in the depression of a germinating net over the top of the jar as a support for seedlings which were raised from seeds sown direct in this small amount of moist sand. Houghland (1947) supported potato plants grown in water culture by growing the plants in a nylon net suspended over the culture solution. In later work,

Houghland (1950) made a tuber compartment attached to the lid of the 30-gallon container. The inclusion of sphagnum was necessary for satisfactory tuber formation and functioned as a thermal insulator. Bolas and Ruck (private communication) used pyrex glass rod frames to support potato tubers " sown " in water cultures. Growth was as good as in sand except that tuber formation was almost suppressed.

Additional support for plants grown in water culture may be obtained by a wire cage, attached to the jar, by the use of dowel rods or glass rods held in a socket in the stopper as suggested by Brenchley (private communication), or by dowel rods held in sockets made from thin sheet metal and wired on to the jar as described by Schreiner and Skinner (1910) and McHargue (1923 a).

Composition of Covers

The material used for covers requires special consideration when micronutrient deficiencies are concerned since the plant is in close contact with the cover, and also, as the result of the saturated conditions, moisture condenses on the covers and may drip back into the culture together with any toxic constituents or micronutrient contamination collected from the cover. A popular method is to prepare covers by moulding them from plaster of paris (leaving holes for the plants) and impregnating them with paraffin wax. This method has been used by Sommer (1931), Brandenburg (1933), van Schreven (1936 a), Stout and Arnon (1939), Hoagland (1941), Millikan (1944) and Wade (1944) for copper deficiency ; by Stout and Arnon (1939) and Millikan (1942) for zinc deficiency ; by Stout and Arnon (1939) for manganese deficiency and by Hoagland (1941) for molybdenum deficiency. Hoagland also used plaster tops treated with asphalt and, in other experiments, glass covers with holes drilled to take the plants. Hardwood covers impregnated with wax have also been used as, for example, by Schropp and Arenz (1938), (1940) for boron deficiency, by Smith and Bayliss (1942) for zinc deficiency, by Piper (1940), (1941), (1942) (and private communication) for deficiencies of copper, manganese and molybdenum, and by Greenwood (private communication) for micronutrient studies with cacao seedlings. Bakelite covers with hardwood plugs previously extracted in hot hydrochloric acid and then waxed were used by Chapman *et al.* (1937), Liebig *et al.* (1943 b) and Vanselow and Datta (1949) for zinc, gallium and molybdenum deficiency tests with citrus. Zinc (and other elements) were detected in the ash of the bakelite. Bakelite was also used by Lehr (1940) for boron deficiency cultures. Plastic covers of " Mipolam " were used also by Schropp and Arenz (1940) for boron deficiency and of " Vinylite " by Evans *et al.* (1950) for molybdenum deficiency. Uralite (? asbestos) covers were used Asana (1945) in a study of iron deficiency.

Composition and Selection of Material for Containers

Plant containers may be made in metal, unglazed or glazed earthenware, glass, silica, plastic or concrete and may also be lined in various ways as, for example, with wax, bitumen, glass, or varnish. For many experiments the use of expensive and fragile materials is unnecessary or impracticable. Sometimes, however, insufficient attention has been given to the influence of the containers and toxic or contamination effects have occurred. The problems of choice of container are similar in sand and water culture methods and may be considered as a whole, although certain types of materials have found greater application for one or other of these methods, and root matting and root corrosion effects are probably more important in sand cultures.

Metal Containers

Metal containers are frequently used in large-scale work on account of low cost and great strength and usually where problems of contamination by metal impurities are not serious. However, Raleigh (1939) found bitumen-painted iron tanks necessary for the study of silicon deficiency in beet, which he was unable to produce in pyrex glass vessels or mason jars. Arnon (1938) considered that large (40 L. or 115 L.) bitumen-painted iron tanks were unsuitable for zinc and copper deficiencies but were satisfactory for those of manganese and

boron and also concluded from the growth of lettuce in these tanks that an additional element in the B7 group (now identified as molybdenum) was necessary. Smith (1943), however, obtained responses to boron, manganese, copper and zinc deficiencies, but not to molybdenum deficiency in bitumen-painted iron tanks. Goldschmidt (1943) has, however, shown that bitumens and asphalts may contain relatively high concentrations of molybdenum as well as of copper. Colwell (1943) used small re-enamelled cannery tins for the sunflower test for boron. The metal containers used were frequently of plain black iron (or mild steel) treated with bitumen paint or asphalt, as for example those used by Reed and Haas (1923 a), Eaton (1931), Hoagland and Arnon (1939), Raleigh (1939), Smith (1943), Davidson (1946), or 50lb. food cans painted with asphalt and provided with drainage used by Kenworthy (private communication). Galvanised containers have also been frequently used, and bitumen or asphalt treatment is then even more necessary. Such containers were used by Reed and Haas (1923) and Haas (1949) for citrus, by Parker and Pierre (1928) and Sommer (1936) for several cereal and forage crops, by Bould (1939) for bulbs and by Brown (1945) for peach. Untreated galvanised containers are quite unsuitable and caused severe toxicity in work by Ehrenberg (1910), Conner (1920) and others. Conner found that paraffin linings were unsatisfactory owing to rapid breakdown and used a protective coating of wood tar.

Pure bitumen paints have been found quite satisfactory as regards freedom from toxic effects in numerous trials by the writer. The possible injurious effects of other paints has been emphasised by Hoagland and Arnon (1939), and these authors also point out that galvanised surfaces are less suitable for paint then black iron owing to scaling of the paint film. Metal containers treated appropriately with asphalt or bitumen preparations appear to be non-toxic and to be satisfactory containers for large-scale cultures and for boron deficiency tests. The painting of galvanised surfaces is considered further in Part Two. Bitumens and asphalt preparations are discussed in relation to cement and wood containers.

Enamelled containers have also been used for culture work, but may give rise to contamination, especially by zinc or boron, as found by Haas (1930), Haas and Klotz (1931) and Trumble and Ferres (1946). Enamelled pots used by Vogel (1929), (1931) were specified as being " boron free," an indication of the likelihood of contamination by boron-containing enamels. Hoagland and Broyer (1936) advised painting enamelled vessels with bitumen even when required only for absorption tests with micronutrient elements.

Cement Containers

Cement troughs or pits have been used for large-scale and tree sand cultures, as for example in the work of Hayward and Long (1941 a, b), Hayward *et al.* (1946), in studies on salt tolerance of peach trees ; or for automatically operated large-scale cultures by Eaton (1936) (1941 a) ; and in long-term experiments by Lagasse (1929) who kept peach trees alive for 19 years in concrete pits holding 22 tons of sand. Cement beds were also used for hydroponic work by Withrow and Biebel (1936) and Withrow and Withrow (1948). The concrete beds noted above were provided with ample drainage and were treated with bitumen or asphalt preparations or emulsions. Details of the correct grades and methods of application of these are specified in papers by Eaton (1936) (1941 a), Withrow and Biebel (1936), Withrow and Withrow (1948) and Davidson (1946).

Useful information on the properties and application of bituminous materials is given by Preston (1947). Withrow (and others cited) emphasised that asphalt or bitumen preparations must be petroleum asphalts in order to ensure freedom from toxic constituents which may occur in coal tar derivatives. Withrow and Withrow (1948) used a simple test to determine whether their preparations were toxic. Seeds were made to germinate in contact with aggregate or filter paper treated with the asphalt to be tested and results compared with a control.

Methods recommended to treat metal or concrete surfaces include application of asphalt dissolved in naphtha or in the molten state without a solvent, but this entails special care not to exceed the flash point. Concrete may be treated either with asphalt emulsions, which are suitable for use on damp surfaces or before the concrete has matured, but care is needed to

avoid " breaking " the emulsion, which renders the preparation useless. Some emulsifying agents may be toxic or may be a source of micronutrient contamination. The other methods can only be used on completely dry surfaces and the first coat should be thin to obtain penetration and to avoid peeling. Withrow and Biebel (1936) and Davidson (1946) suggested lining metal or wood tanks with roofing felt before treatment with hot (fluid) oxidised asphalt. Metal may also be painted with bitumen or asphalt without lining as described in the Section on water and in Part Two. Hoagland and Arnon (1939) pointed out that cement tanks need prolonged leaching before use even if painted, which is in any case desirable. Drainage is effectively provided by laying porous bricks over gutters, Hayward *et al.* (1946), or gravel above porous aluminium silicate brick over gutters, Eaton (1941 a). Cement containers when properly treated are apparently non-toxic, but would not be suitable for calcium deficiency.

Wood and Paper Containers

Wood tanks have been used for sand cultures by Hoagland and Arnon (1939), Arnon and Hoagland (1940), and Arnon and Grossenbacher (1947). Hoagland and Arnon point out that redwood may be toxic unless well leached and then painted with a bitumen preparation. Davies (private communication) used waxed card containers in a study of molybdenum deficiency and detected microgram quantities of molybdenum in them.

Plastic Containers

Schropp and Arenz (1940) used flasks of " Mipolam " (a polystyrol resin) for boron deficiency experiments with gramineæ (notably resistant subjects). Taubock (1942) used containers made of a synthetic plastic for work on the aluminium nutrition of pteridophytes. Plastic containers have been examined by the writer and the results are given in Part Two. Polythene has been found to be a promising material for use in the study of deficiencies of iron, manganese, boron and molybdenum. Polythene is relatively expensive, but it may be of especial value for lining large tanks or similar vessels and can be " welded " in a stream of hot nitrogen. The high price makes it compare unfavourably with pyrex or hysil glass for smaller containers, except that polythene containers are practically unbreakable, which is an additional advantage for sand culture work.

Samples of polythene and also cellulose acetate have been analysed by the writer with the following results :—

		Fe	Mn	Cu	Zn	Mo
Polythene						
Ash 0·045%	% in Ash	2·0	0·25	0·5	1·0	Not
	p.p.m. in original ...	8	1	2	4	detected
Cellulose acetate						
Ash 0·078%	% in Ash	0·085	Not detected	2·1	0·17	0·025
	p.p.m. in original ...	0·7	—	16	1·2	0·2

Unglazed Clay Containers

Unglazed clay pots were used for early work on the nutrition of fruit trees by Wallace (1922, 1924, 1930), Davis (1930), Davis *et al.* (1934), by Beard (1932) for hop nutrition cultures, by Tyner (1935) for the study of potassium uptake in drip culture experiments with buckwheat and clover, by Woodman (1940 b) in a study of the nutritional requirements of turnips and lettuce, by Clark (1941) in a study of the effects of pH and nitrogen supply on the growth of strawberry and by Hewitt (1944), (1945) in survey experiments for the recording of visual symptoms of mineral deficiencies in vegetable crops. In this work the clay pots were treated by immersion in molten paraffin wax to impregnate the porous clay. This treatment was designed to render the pot impermeable to water and unreactive as a possible source of nutrients to the plants. Wallace (1930), Beard (1932) and Hewitt (1944), (1945) found that calcium deficiency was often difficult to produce when using these pots. Woodman (1939 c), 1941 a) observed that the paraffin wax coating was broken down after a season's use and this

breakdown was confirmed by the writer. It was shown (Hewitt unpublished) that breakdown of paraffin wax films was a biological process and associated with a group of fungi identified as *Stemphylium macrosporoideum* and species of *Penicillium* and *Aspergillus*.* Incorporation of a number of organic mercurial fungicides in the wax caused unusual effects. At low concentrations (1-10 p.p.m. by weight) a *significant* stimulation of fungal attack on the wax and of plant growth (fresh weight of flax and barley) occurred in the impregnated pots, whilst breakdown was largely prevented at a concentration of 100 p.p.m., but slight depression in plant growth sometimes also occurred. It was also found that if heavily waxed clay pots were allowed to stand with nutrient solution in them, the wax film disintegrated in 4-6 weeks unless formalin was also included in the solution, when no breakdown occurred. Penetration by roots through the wax film where minute air bubbles had caused pin holes also occurred and a similar observation was made by Salm-Horstmar (1851) when he used waxed glass vessels. Roberg (1928) also found that wax linings could serve as a carbon source for micro-organisms. The breakdown of the paraffin wax film coincided with the marked visual recovery of plants showing symptoms of boron or of calcium deficiency, Hewitt (1946 a), (1947 a), and this recovery was traced to the contamination by the clay pot. Such pots can supply all the calcium or boron required by many plants to complete their life cycle normally. Bishop (1928) found manganese in the hydrochloric acid extract of unglazed pots but not in that from glazed ones.

Plain clay pots are not, however, always unsatisfactory for nutritional studies, even those relating to micronutrient deficiencies. Thus Miller (1928) produced acute manganese deficiency in tomato, maize, wheat, barley, cabbage and tobacco plants grown in sand culture in untreated clay pots.

MacVicar and Struckmeyer (1946) found varnished clay pots satisfactory for the production of boron deficiency in soya bean. In the writer's experience, synthetic resin varnishes are not suitable for the treatment of clay pots, as plants grown in pots treated thus have not shown severe symptoms or have shown recovery after 3-6 weeks. Catalysed cold-setting lacquers have proved more promising but are brittle and liable to shrink ; splits then occur and the pot is no longer reliable.

A convenient and simple way of making clay pots unreactive is to use a bitumen or asphalt lining. This has been done by Behrens (1928) and extensively at Long Ashton by Hewitt (1946 a, b) (1947 a, b) (1948 a) (1949 a, b) for a great variety of experiments. The method is described in Part Two. Bitumen-painted clay pots are satisfactory for the study of all macronutrient deficiency and excess problems and also for work with iron, manganese or boron deficiency, but not with molybdenum deficiency. It is not certain how long the bitumen lining remains intact. Many pots emptied at the end of a 6 months' experiment often appear to have the paint film intact, but others show signs of breakdown of the paint, and fruit bushes grown without calcium for three years in painted pots appear to have obtained relatively small supplies of calcium during the second and third years. The application of bitumen paint is worth while whenever clay pots are to be used in sand culture experiments, since not only is contamination greatly reduced but the opposite effects of nutrient absorption which may occur in unpainted pots are also avoided. Pollard *et al.* (1943 a, b, c) investigated the effect of clay pots on the availability of nutrients in soil. Marked depletion of nitrogen and phosphorus was observed due to evaporation and subsequent absorption of the solution into the pottery, which also introduced calcium and potassium.

The use of clay pots is justified, therefore, only if thoroughly treated with a bitumen preparation. Under these circumstances clay pots can be safely used for a wide variety of culture experiments, over a limited period of probably 6-12 months.

Glazed Containers

Glazed clay containers are generally assumed to be superior to unglazed ones and suitable for the study of all macronutrient deficiencies and also those of some micronutrients. They are, however, considerably more expensive and the composition and acid-soluble fractions

* These fungi were identified by Mr. R. W. Marsh, whose help is gratefully acknowledged.

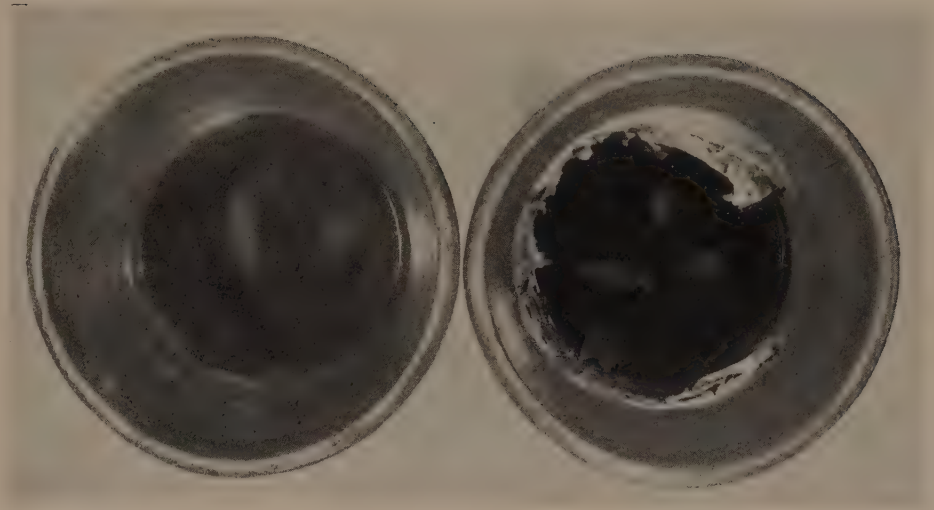


PLATE A

Biological breakdown of waxed lining in clay pot.

Left : **After holding nutrient solution and formalin 4 weeks.**
Right : **Effect in absence of formalin.**

[*To face page* 20

PLATE B

Effect of container on production of iron and manganese deficiencies in oat.

Left : **Without manganese.**

Right : **Without iron, here the glazed container has proved unsatisfactory.**

of the glaze may vary widely. A number of glazed sample pots were examined by the writer by exposing the cleaned surface to the steam from a jet of boiling 20% hydrochloric acid for 1 hour. The condensed vapour was collected in pyrex glass and a " blank "determination was also carried out. The following micronutrient elements were detected (figures are maximum values as μg per sq. cm. per hour) :—

Iron 7, manganese 0·008, boron 22, zinc 0·4, molybdenum 0·003.

The samples generally fell into one of two groups, one high in boron, the other high in zinc. Iron and molybdenum often showed parallel variation. One sample was outstanding* and yielded minimum quantities of these elements. The lowest amounts extracted were usually 2-4% of the figures given above and as little as 0·5% of the maximum for boron. The glaze used in many pots is a mixture of boron and zinc oxides.

Glazed containers (with a drainage hole) have been widely and successfully used for studies with macronutrients, pH changes and absorption problems, as for example by Parker (1927), Gregory and Crowther (1928), Gregory and Richards (1929), Richards (1932, 1938), Richards and Templeman (1936), Clark (1933), Spencer and Shive (1933), Davidson and Shive (1934), Nightingale (1934), Eaton (1936), (1941 a), Addoms (1937), Wadleigh *et al.* (1937), Chapman and Liebig (1938), Cullinan *et al.* (1938), Mitchell (1939), Woodman (1939 a), (1940 a), (1941 a, b), (1944 a, b), Batjer and Degman (1940), Shive and Robbins (1942), Gauch and Wadleigh (1943), Lineberry and Burkhart (1943), Guest and Chapman (1944), Robbins (1946), Roy and Gardner (1946), Houghland (1947). Glazed vessels have also been used in a number of water culture experiments as for example by Colby (1933), Eaton (1941 a), Guest and Chapman (1944), Asana (1945).

Bishop (1928) and Hageman *et al.* (1942) used glazed acid-proof vessels for the production of manganese deficiency in several plants in sand culture and MacHargue and Calfee (1932 a) found glazed vessels were satisfactory for manganese deficiency studies with *Lemna* in water culture. McHargue and Calfee (1932 b), Chandler (1941) and Reeve and Shive (1944) found that glazed vessels were also suitable for the study of boron deficiency in lettuce and tomato.

McHargue (1923 c), however, pointed out that inferior quality glazed containers were unsatisfactory for use in nutritional studies. These containers caused contamination by manganese and were also able to retain appreciable quantities of nutrients after use owing to imperfections in the glaze. Good acid-proof stoneware was, however, satisfactory. Robbins (1946) also emphasised the importance of high-quality glaze. A standard silica glazed vessel used at New Jersey was stated to remain free from " crazing " and to be satisfactory for production of deficiencies of iron, manganese and boron. Stout and Arnon (1939) found that glazed vessels caused contamination by metals detected by the dithizone test, in spite of a bitumen coating.

Glazed containers of a standard pattern commonly used for nutritional experiments have been tested at Long Ashton ; they were found to be satisfactory for work on manganese or boron deficiencies, but *useless* for the production of iron deficiency in a number of crops including oat, tomato and clover grown in sand culture. Comparative trials with other containers including bitumen-treated unglazed clay pots showed that the sand and other points of technique were not at fault (Plate B).

It may be concluded that whilst glazed containers are superior to unglazed ones in certain respects and satisfactory for work with macronutrients and related problems they are by no means faultless when micronutrients are considered—even the more common ones such as iron. It seems that manganese deficiency may be readily produced in many glazed types of vessels but their suitability for boron and other micronutrient deficiencies is less certain. The high cost of best-quality glazed vessels may make their use unjustified where bitumen-treated containers are equally suitable, and their uncertain nutrient status combined with high cost makes them compare unfavourably with the heavy-weight pyrex glass containers of similar capacity described in Part Two. Some of the responses due to the container are illustrated in Plates B & C.

* This sample was a semi-porcelain type developed by Messrs. Hathernware Ltd., Loughborough, to whom thanks are due for the supply of samples for test.

Glass Containers

Glass vessels have been very widely used, especially in water culture work, just as clay pots have been used for sand culture. It is unnecessary to review in detail the use of glassware in water culture experiments dealing with problems relating to macronutrient elements. For much of this work the familiar " Mason " jars holding usually 1 or 2 quarts (about 1·2-2·4 L.) have been used, for example by Sommer (1926), Shive and Robbins (1942). Also popular are glass bottles holding from 250 to 1,000 ml. as used by Tottingham (1914) and many others since that time in the United States and by Brenchley (private communication) at Rothamsted. Milk bottles have also been found useful by Brenchley as they are strong, convenient in shape, easily sterilised and cleaned and free of contamination from boron. Trumble and Ferres (1946) have shown, however, that certain types of glass may cause significant contamination by potassium.

Although glass is considered to be relatively unreactive it is doubtful if any single type of glassware is suitable for work with deficiencies of all the known micronutrient elements, and certain types of glass as shown below may give rise to serious contamination, for example, by zinc, boron and possibly other elements. Containers made of soft glass have been widely used for the study of boron deficiency. van Schreven (1934 a) and Löhnis (1937) found ordinary jam jars suitable for this purpose. Haas and Klotz (1931) and Löhnis (1937) pointed out the beneficial effect of continued use on " seasoning " the glass. Schropp and Arenz (1938) also used containers seasoned by many years' work. The jars used by van Schreven were waxed internally with paraffin wax. Mason jars used for the study of aluminium deficiency by Sommer (1926) were also either waxed or varnished. Samuel and Piper (1929) and Arndt and Hoffman (1937) used waxed glass vessels for manganese and copper deficiencies respectively. Although soft glass vessels are suitable for work with boron deficiency, those made from pyrex glass may not be. Pyrex glass has a high boro-silicate content and under slightly alkaline conditions appreciable amounts of boron may come into solution. McHargue and Calfee (1933) showed that not only is powdered pyrex glass an excellent source of boron in sand culture but that at concentrations of 0·5% (by weight) in the sand pyrex glass may even cause marked boron toxicity. Muckenhirn (1936) confirmed the effectiveness of powdered pyrex glass as a source of boron in sand culture. Chandler (1941) used pyrex glass vessels for boron deficiency tests in water culture. Pyrex glass would presumably be unsuitable as a material if silicon deficiency were to be studied, as found by Raleigh (1939), although pyrex glass was used for a similar purpose by Lipman (1938), and waxed glassware was used for this purpose by Wagner (1940).

Whilst pyrex glass is probably unsuitable for work with boron deficiency it is the most widely recommended type of glass for use when deficiencies of any of the other known micronutrients are involved, Stout and Arnon (1939), Piper (1944, private communication). Pyrex glass has been successfully used for production of micronutrient deficiencies and especially of copper in higher plants by Lipman and MacKinney (1931), Sommer (1931), Brandenburg (1933), van Schreven (1936 a), Stout and Arnon (1939), Hoagland (1941), Millikan (1944), Smith (1943), Wade (1944), Hewitt and Jones (1951), of iron in bacteria by Waring and Werkman (1942), of manganese by Stout and Arnon (1939), Smith (1943), Pearse (1944), of zinc by Sommer and Lipman (1926), Chapman *et al.* (1937), Stout and Arnon (1939), Millikan (1942), Smith and Bayliss (1942), Smith (1943), and of molybdenum by Arnon and Stout (1939), Hoagland (1941), Hewitt and Jones (1947), (1949), (1950), Mulder (1948 a) Vanselow and Datta (1949), and Evans *et al.* (1950).

In view of the high efficiency of pyrex glass for use in most micronutrient deficiency cultures it is of interest to note the results obtained with other types of glass and to compare vessels made of pyrex glass with those of pure silica.

Bertrand and Javillier (1912 a) found that manganese was introduced into the culture solutions of *A. niger* during sterilisation of the Jena glassware. A similar finding was reported by Mazé (1914) for manganese during sterilisation of the glassware containing the culture fluid. Mazé (1914) also reported that zinc deficiency was more severe to the extent of a 40% reduction in yield when old (well-used) flasks of Bohemian glass were used as compared with

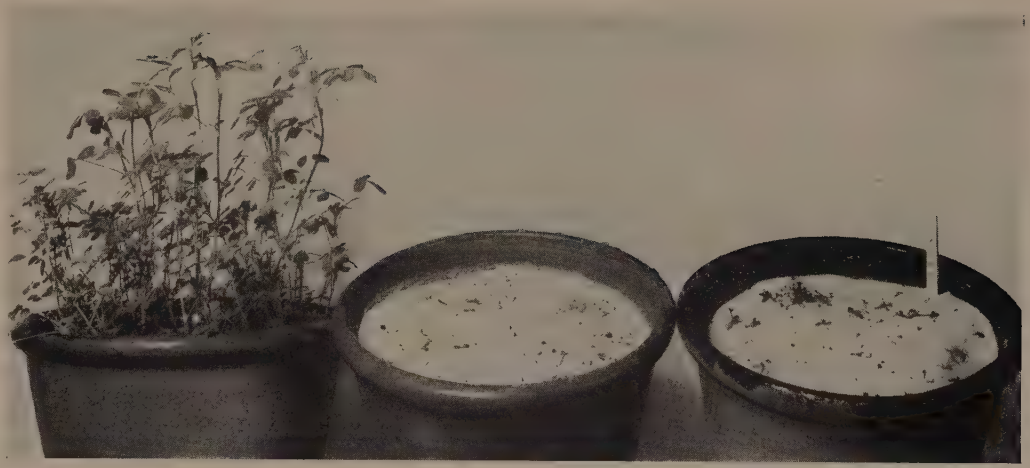


PLATE C

Boron deficiency in lucerne and effect of container.

Left : **Varnished clay pot.** *Centre :* **Double-waxed clay pot (prior to possible recovery effects).**
Right : **Bitumen painted clay pot.**

[*To face page* 22

new ones. Javillier (1914) pointed out that glassware may frequently be a cause of error in micronutrient deficiency work and compared the yields of *A. niger* grown without zinc in vessels of different types of glass with the following results :—

(4 days' incubation). Yields in g. (Javillier 1914)

	Jena Glass		Kavalier Glass		Quartz	
	(a)	(b)	(a)	(b)	(a)	(b)
—Zn	1·861	1·365	0·352	·308	0·291	·219
+Zn	1·836	1·762	1·780	1·823	1·624	1·645
Difference	— ·025	+ ·397	+1·428	+1·515	+1·333	+1·426

Javillier (1914) was able to isolate 2·5 mg. Zn from 6 L. of distilled water evaporated in a Jena glass flask, i.e. 0·05 mg. Zn per 125 ml. of culture solution. Sommer and Lipman (1926) and Steinberg (1919) also found that zinc contamination came from Mason jars or " ordinary " glass as compared with pyrex glass and could account for almost the total needs of some organisms. Walkley (1942) found that a resistance glass caused considerable zinc contamination compared with pyrex glass, but Piper (private communication) has found that a few types of " pre-war Continental " resistance glass are relatively free from zinc contamination. A similar inferiority of Jena glass as compared with quartz was noted by Gollmick (1935) in a study of micronutrients including zinc needed by *A. niger*. Steinberg (1935) (1936) compared the effectiveness of quartz and pyrex glass flasks for the production of deficiencies of iron, manganese, copper, zinc and molybdenum in *A. niger* and found no apparent difference as judged by dry weights of mycelia produced. Deficiencies of copper, manganese and molybdenum have also been produced by Piper (1940), (1941), (1942) in accumulator jars of unspecified glass. Hewitt and Hallas (1951) have shown conclusively that hysil glass is also entirely suitable for molybdenum deficiency in *A. niger* and it may be concluded that pyrex or hysil glass vessels are reliable and convenient for the production of deficiencies of the known micronutrients except boron. The use of silica does not seem justified at present for use in experiments with higher plants or fungi on account of high cost compared with little or no gain in efficiency, unless as yet unrecognised micronutrients are sought.

An interesting application of glass for containers was the use by Thomas *et al.* (1943 a) of plate glass as a lining for the concrete tanks installed in the large-scale air-conditioned greenhouse units. These tanks were used both for holding the sand and storing the nutrient solutions.

Cleaning Containers (with special reference to glassware)

The removal of traces of heavy metals from glassware is not always complete if water or detergents only are used. It is generally considered that acids and (or) some steaming treatment are necessary for this purpose. Many workers, for example Piper (1942), Hageman *et al.* (1942), Millikan (1944), Wade (1944), cleaned containers by treatment with 1 : 1 or concentrated hydrochloric acid followed by rinsing with water (usually glass distilled). Bishop (1928) immersed glazed containers in boiling 1 : 3 hydrochloric acid as a cleansing method. Autoclaving or steaming has been used in addition to acid treatment by McHargue and Calfee (1932 a), Smith and Bayliss (1942), Brenchley (private communication), Hewitt and Jones (1947), (1949) and unpublished. Hewitt and Jones first washed the glass containers in hot water with a detergent ; this treatment was followed by immersion in concentrated hydrochloric acid for about a half an hour and then steaming for a similar period before rinsing, or alternatively as for the most refined experiments (for example with micronutrient deficiencies in *A. niger*) the glass vessels or flasks were " steamed " over a jet attached to a flask of constant boiling hydrochloric acid. A more elaborate procedure was used by Roberg (1928) and Waring and Werkman (1942). The former used in turn, spirit (alcohol), acid and water washing and finally autoclaving of the completely filled flasks, which was considered especially important. The latter workers used in turn soap and water, alcohol, water, nitric acid, water and finally

autoclaving at 20-30 lb. pressure ; this last step was regarded as essential for the removal of traces of absorbed iron compounds. Piper (1942) rinsed culture vessels (already cleaned) with 1% acetic acid, and glass distilled water immediately before use. Vanselow and Datta (1949) used soap, alkali and acid to clean containers for molybdenum deficiency tests. Hutner *et al.* (1950) suggested including a suitable chelating agent in the cleaning solution to aid in removing precipitated compounds.

The use of acid, and steaming or autoclaving, or the use of an " acid steam " from constant boiling acid appears to be the simplest reliable procedure for precise work. It has been proved by Hewitt (unpublished) to remove molybdenum (known to have been added) to a level less than 10^{-4} μg.

Multiple Compartment Containers and " Split Root " Methods

It is sometimes desirable to enable a particular element to enter a plant without interference due to precipitation or to antagonism by other elements, or to test the effects of one group of plants on the growth of others when the roots are separated by a membrane, or to study the effectiveness of different root systems, for example adventitious and primary roots, for the absorption or distribution of particular nutrients to the rest of the plant. Anderson (1944 a) has pointed out that in two of these respects, such experiments are analogous to injection techniques (Roach W.A. (1918) Imperial Bureau of Horticulture and Plantation Crops Tech. Commun. 10) and refers to the work of Frank in 1893 as one of the earliest attempts to use a " split root " technique. The methods considered here include : (a) multiple containers in which separate plants may be grown ; (b) multiple-compartment containers in which the roots can grow from one section to the next ; (c) " split root " devices in which the root system is partitioned between separate containers.

Method (a) : Multiple containers were used by Lipman (1910) (1912) to study the effect of leguminous crops on associated non-legumes with particular regard to the excretion and utilisation of nitrogenous compounds. An outer 5-gallon glazed pot (holding 80 lb. sand) enclosed a concentric, smaller pot holding 20 lb. sand. This pot was either glazed (and thus impermeable) or unglazed and permeable to soluble materials. A legume was grown in one compartment and a non-legume in the other. The results were inconclusive and the experiments were not developed further.

Method (b) : In these methods the roots of the culture plants develop in one compartment and grow through the base of it into another. They are limited either to sand culture or to the use of a solid medium in the upper compartment and a solid or liquid medium in the lower one, but avoid the splitting of a root system by mechanical means. Went (1943 a) grew tomato plants in a basket supported over a vessel holding solution. The plants produced roots that developed in peat, which enclosed saturated air in the basket, and also grew into the solution below. This arrangement was found to eliminate the iron deficiency chlorosis that occurred when the plants were grown with the whole root system fully immersed in solution even when aerated. Replacement of the peat by sand gave inconclusive results. These experiments were repeated and extended with flax to include the use of alternating solution (with or without phosphate) by Richards (1945, 1946 unpublished). The sphagnum moss was retained in a silver wire basket above a glazed pot. The results did not confirm the promise claimed by Went except as a temporary effect during the seedling stage. In spite of the slight reduction in chlorosis the use of the Went technique caused a depression in yield compared with normal water culture controls.

A similar double-compartment pot technique has been devised by Anderson (1944 a) for soil and solution cultures and is applicable to other solid media including sand. The basket compartment has a 0·25 inch mesh screen bottom and the solid medium is held by glass wool which allows the roots to penetrate to the solution below held in a glass jar for purposes of inspection and darkened by a paper cover. The basket is made of black iron, tinned and lacquered in an oven, and is supported loosely in the vessel by a flange around the top.

Davidson (1944) used a double-pot technique with peach trees in which part of the root system grew in the upper compartment and the rest was insulated within a wide glass tube

TABLE 4

Comparative Data for Mechanical Analysis of Sands Used in Culture Experiments

Reference	Crops grown	Remarks	Grading in terms of mm. particle size. Fractions as per cent. of total										
			>2 mm.	2–1	1–·5	·5–·4	·4–·3	·3–·25	·25–·2	·2–·15	·15–·125	·125–·1	<·1
Davidson (1946)	Rose	6 and 12 inches deep. Intermittent surface application			·8	99%	·3						
Eaton (1931)	Several crops	Surface irrigation, beds 11 inches deep				←			100				→
Eaton (1936)	Several crops Fruit trees	Surface irrigation, beds 13 inches deep Beds 6 feet deep			2		←2	5*→	54	13	6		
Eaton (1941 a)	Several crops	Surface irrigation, pot cultures					←5	0→					
Gauch and Wadleigh (1943)		Surface irrigated, pot cultures					85						
Gile and Fenstell (1943)	Millet	*a*. Induced wilting *b*. Satisfactory *c*. Too compact	27	68 16	64	←	17	→	←	84	→		13
Hewitt (1945)	Several crops	10-inch pot cultures. Intermittent application	·15	1·0 5·0	86·6 90·5	←			12·3 4·5				→
Hewitt (1946 a, b)	Ditto	Ditto	0·2	12·4	83·7	·63		2·4	·4	←	·2	→	
Hewitt (1948 unpublished)	Tomato, beet, oat and potato	10-inch pots. Intermittent application	0·1	0·2	4·4	2·0		85·3	5·0	←	2·8	→	
Hicks and Tincker (1944)	Carnations	Sand beds	25	←	30	→	←	40	→	←	5	→	
Hoagland (1919)	Barley	Undrained vessels, excess liquid removed by siphon							60	26·7	← 9·7	→	3·5
McCall (1916 a)	Wheat	Small undrained vessels holding 1·5 kg.		·14	48·6	←	26·4	→	←	22·9	→		1·5
Reed and Haas (1923 a)	Citrus	Large vessels			←	·3	→	7·85→	54·1	2·16	←	16·1	→
Robbins (1937)	Tomato	Drip cultures 8–9 inches deep Ditto	2·3	35·0 41·7	63·7 53·3	←	1·3 2·7	→					
Robbins (1946)	Several crops	Ditto	5·1	29·6	51·1	←	7·7	→	←		6·5	→	
Shive (1918 a)	Buckwheat	Undrained pot. Maintained 15% moisture	·6	5·6	30·8	←	53·9	→	7·36	←		1·7	→
Thomas *et al.* (1943 a)	Several crops	Large air-conditioned cultures 2·5 feet deep			·6–·5 100								
Wallace (1924)	Fruit crops	Pot cultures. Intermittent application	←	7·8	→	←	90·1	→	←		2·1	→	
Woodman (1939 b *et seq.*)	Vegetable crops	Ditto						2·1	44·9	21·6	24·7	2·9	2·7

* ←25→ indicates 25% between the limits shown.

[*To face page* 24

and led into the lower part. This procedure thus presented features common to methods (b) and (c). The upper compartment was a varnished clay pot, with a 1-inch diameter glass tube, sealed by the lower end into the central drainage hole. The tube was 7 inches long and thus projected above the level of the sand in the upper pot. A new drainage hole was made at the base in the angle with the sides and oriented to be above the side tubulure of the lower glazed pot. A tube was led from the upper drainage hole down through the sand in the lower pot and out through the tubulure of the lower pot (but the reason for this devious path was not made clear). The peach seedling was planted in the upper compartment in such a way that the lower region of the root system was inserted through the glass tube and grew into the sand in the lower compartment. No leakage between the two sections occurred and evidence of translocation of potassium in both directions was obtainable.

Method (c) ; In this method the root system is divided mechanically or split symmetrically and is the most widely used. With this technique unequal effects due to unilateral uptake (or injury) and subsequent distribution of the effects to the aerial parts are common, as was found for example by Trenel and Alten (1934) for aluminium toxicity in maize, Martin (1935) for iron deficiency in sugar cane, and McMurtrey (1937) for macronutrient deficiencies in tobacco. Cereals and other monocotyledons have been used in many " split root " experiments as noted above and also by Gile and Carrero (1917), (1921) for rice and maize, by Krassovsky (1926) for wheat, barley and rye, by Wright (1937) for barley ; by Wagner (1940) for maize, rice, oats, barley, and also tobacco and tomato ; by Eaton (1941 b) for maize and also tomato ; and by Anderson (1944 a) for oats. Tomato plants are also well adapted to such experiments and in addition to the work cited above they have been used by Haynes and Robbins (1948), whilst Tyner (1935) used buckwheat and clover. The main consideration is the suitability of the root system for division into symmetrical and approximately equal regions. The tomato has an extensive fibrous system arising from a diarch arrangement in the seedling stage which can if necessary be split upwards towards the stem, Haynes and Robbins (1948). McMurtrey (1937) also split the root system of tobacco up to the stem in order to avoid capillary " wick " effect caused by the intervening fibrous roots between the two culture vessels. The roots were sterilised in silver nitrate solution. The stem splitting method was not, however, considered satisfactory and in later experiments the " wick " effect was overcome by cutting away the roots that developed in the intervening region.

Although most experiments with split roots have been carried out in water culture, the method is applicable also to sand culture as shown by Anderson (1944 a) who partitioned the root system of oats suspended from a small bridge over two adjacent containers (one holding sand and the other soil, and by Tyner (1935) who used a central container holding bentonite inserted in an outer vessel filled with sand. Anderson (1944 a), however, has pointed out that arrangements resembling the one described are unsatisfactory for tap-rooted plants, especially if the tap root exhibits special absorption features. Martin (1935) combined sand and water culture in a split root experiment with sugar cane in which the lower fibrous roots were grown in iron-deficient sand cultures whilst nodal adventitious roots were trained into flasks of solution from which iron could be absorbed. Marked bilateral green and chlorotic effects in the chlorotic leaves were produced in these experiments. Richards (1945) used an analogous technique with flax, but here the two root systems both developed in an aqueous medium.

In most experiments two vessels have been used, but in some work the root systems were divided into three or four sections. Thus Gile and Carrero (1921) grew maize with roots divided into three parts and each flask contained all, two or only one of the nutrients from a three-salt type solution. Growth was reduced even when all the essential nutrients were supplied, but divided between the separate flasks. Eaton (1941 b) was able to partition the root system of maize or tomato plants into four compartments. These were square section 2-pint or 1-gallon cans arranged in a square so that the roots were inserted through a hole in each, bored in the corner of the inside angle at the top of each can. Krassovsky (1926) used two D-shaped vessels which held 850 ml., arranged back to back to eliminate the " dead " space between the two vessels.

SOLID ROOTING MEDIA

Sand is the usual rooting medium used for experimental work in artificial cultures. The grading, analysis and properties of sands used are discussed below. In some of the early experiments several solid media were tried. Thus Salm-Horstmar (1849), (1851), experimented with quartz, silica sand, river sand, rock crystal, broken porcelain, pure calcium carbonate, silicic acid and sugar charcoal as rooting media for oat. Boussingault (1886) used carbon and silica sand as rooting media, Hall *et al.* (1914 a, b) grew lupin and barley in sand of different grades, silt, and kaolin as well as in water alone. A few examples of the use of other rooting media may be mentioned here. Cibes *et al.* (1947) used a " pea " gravel with calcareous contamination for studying mineral deficiency effects on vanilla. An aggregate called " Haydite " has been frequently used for irrigation work in large beds by Withrow and Biebel (1936), and Laurie and Kiplinger (1940). " Vermiculite " or " Zonolite," a heat-processed porous mineral mica, has been used as a good rooting medium for lilies by Woodcock (1946). Whittenberger (1945) used 30-mesh " Crystallite," a non-toxic granular methyl methacrylate polymer in a study of silicon deficiency. Evans *et al.* (1950) used $\frac{1}{4}$-inch pyrex glass cullet mixed with glass wool (to improve moisture retention) in a study of molybdenum deficiency of alfalfa.

TABLE 5

Approximate Correspondence between Particle Size and Standard Sieve Apertures

U.S. Standard Sieve	I.M.M. Standard Sieve	mm.	inch
—	10	1·25	1/20
20	—	0·84	1/30
30	20	0·625	1/40
40	30	0·42	1/60
50	40	0·31	1/80
60	50	0·25	1/100
70	60	0·21	1/120
80	70	0·18	1/140
—	80	0·155	1/160
100	—		
—	90	0·14	1/180
120	100	0·125	1/200
—	120	0·105	1/240
200	—	0·07	1/360

(*N.B.*—I.M.M. value × mm. aperture = constant approx. 12·5).

Particle Size of Sand

MECHANICAL ANALYSIS

The data in Table 4 show the mechanical analysis of sands used in culture work. The approximate grading is given in mm. to facilitate comparison on an equivalent basis of particle sizes, and has been converted where necessary from mesh gradings as shown in Table 5. The crops grown in the experiments are stated together with the appropriate references. In most instances more than 80% of the sand particles were retained by 0·5 mm. mesh sieve. That used by Eaton (1936) was somewhat finer and was from 0·4-0·15 mm., but in a subsequent paper Eaton (1941 a) the grade was somewhat coarser, viz., 0·4-0·25 mm. Thomas *et al.*

(1943 a) used a sand closely graded from 0·5-0·6 mm. The sand used by Hoagland (1919) Reed and Haas (1923 a) and by Woodman (1939 b) *et seq.* was considerably finer and ranged from 0·25 mm. down.

Choice of Particle Size

Gile and Fenstel (1943) compared the growth of millet in three grades of sand (Table 4) and found that, while the coarse grade was liable to produce wilting due to insufficient water retention, the finest grade was too compact to permit of healthy root development of this crop. Hall *et al.* (1914 a, b) compared the growth of lupins and barley in coarse sand 0·2 mm. and fine sand 0·2-0·04 mm., in silt, in kaolin and in water and found that the order of best growth was coarse sand, kaolin, fine sand or silt, water. The reason for the superiority of kaolin over fine sand as a medium is of interest ; growth in fine sand and silt was relatively poor. The observed order was attributed partly to the degree of aeration obtained. Evans (private communication) found that cacao required a coarse sand and grew well when the particle size was 3-5 mm.

Differences between growth produced by different grades of sand may be ascribed to relative water-holding capacity, aeration and compactness, which affects the ability of plants to make healthy root development. Eaton (1936) has pointed out the importance of avoiding a grading giving wide range of particle size, as this tends to produce a medium with insufficient pore space which is filled by the smaller sized particles, especially if the proportion of this fraction is comparable to that of the larger particle size. Evans (1950) also strongly emphasised the need to avoid mixed particle sizes for sand used to root cocoa cuttings owing to packing and lack of pore space. Gauch and Wadleigh (1943), however, used sand which contained 85% between 20 and 100 mesh, i.e., between 0·8 and 0·15 mm. in irrigated cultures for the growth of several crops.

Grading should bear some relation to the depth of sand used. Davidson (1946) has emphasised this point and states that shallow beds require coarser grading than deep ones. This is partly explained by the work of Lebedeff (1927), who showed that, if a long column of sand is saturated and allowed to drain, the moisture content is constant to a certain depth (equal to that produced by centrifuging) and increases sharply to a maximum value towards the base. In columns up to 20 cms. in length the moisture in the surface layers is equal to the saturation value but falls from 9-2% of this value when between 30 and 50 cms. long. Davidson found that rose trees grew better in 6 inches depth of 20-50 mesh sand (1·0-0·3 mm.) than in a 12-inch depth, but that growth in the latter was greatly improved by covering the surface with a thin layer of fine sand. Deep cultures (2·5-6 feet) may be filled with sand graded between 0·2 and 0·5 mm. in order to maintain sufficient moisture in the upper regions. Shallow cultures usually have sand graded between 0·2 and 1·0 mm.

The work of E. J. Winter (referred to in the Section on containers) showed that young cauliflower plants in sand in 20-inch deep pots wilted frequently unless watered several times daily, whereas this was unnecessary in 12- or 10-inch pots, owing to the higher water table, which was more important than total moisture content for the small plants.

Cultures supplied with continuous renewal of the nutrient solution may require a somewhat coarser sand than those where solution is given intermittently, since in the former water supply is not limiting but aeration may easily become so. In the writer's experience sand with a grading of 80-90% between 1·0 and 0·5 mm. provides a satisfactory medium for the vigorous growth of a wide range of crop plants when given nutrient solution intermittently, but a more finely graded natural white sand with 85% between 0·4 and 0·25 mm. has also permitted excellent growth in tomato, oat and other crops. In a comparative experiment with four crops it was found that the finer sand produced higher yields of fresh weight at harvest and earlier fruiting in tomato. Germination and early growth of oat and emergence of potato shoots were slightly delayed in the finer sand although total growth was greater. Oats grown in the finer sand were considerably less infected by mildew than those grown in the coarser sand. The data are given in Table 6. The sand was leached with 2% cold hydrochloric acid before use and the technique was as described in Part Two for macronutrient studies.

TABLE 6

Yields of Crops (as fresh wt.g.) Grown in Two Grades of Silica Sand (Hewitt unpublished)

Crop	A (Coarse Sand)	B (Fine Sand)
OAT		
Straw and grain	699	1191
SUGAR BEET		
Whole plants	3823	3966
POTATO		
Whole plants and tubers	3478	4161
TOMATO		
Leaf and stem	1501	1634
Fruit	2683	5252
TOTAL	4184	6886

It may be concluded that a particle size between 2 and 0·5 mm. is suitable for drip culture work; and between 1 and 0·2 mm. for intermittent application when containers holding from five to fifty kg. sand are used.

Water retention by sand is a function of particle size. This is illustrated by data of Shive (1920 a). The water retention of sands of different grading was as follows :—

Grading	1·5-1 mm.	1-0·5 mm.	0·5-0·32 mm.	0·32-0·25 mm.
Water retention (%) ...	26·8	30·2	32·4	37·6

The percentage saturation for satisfactory growth depends on the depth of sand and on the grading. Thus Thomas *et al.* (1943) used 2′ 6″ deep beds and found that sand graded between 0·5-0·6 mm. retained only 11·4% moisture as compared with the data found by Shive—above—using small pots. Similar comparisons are seen to hold from the work of Lebedeff (1927). Large beds of Monterey sand 9 inches deep used by Arnon and Grossenbacher (1947) retained only 9% of moisture. Greenhill and Page (1933) found 13% moisture satisfactory for growth of rye grass in a mixture containing 90% sand, 6% Bentonite, 4% calcium carbonate. Shive (1920 b) grew wheat in small sealed pot sand cultures with varying moisture content. Growth at 60% saturation was better than at 40% or 80% saturation. Bakke and Erdman (1923) found that 11% moisture (about 40% saturation) was too low for wheat in sealed cultures. Wallace (1924) and Waltman (1931) maintained fruit trees and strawberries in sand culture with 20% moisture—about 80% of the saturation capacity. McCall (1916 a) and Shive (1918 a) maintained wheat in undrained sand cultures with a moisture content of 15% (which was 60% of the saturation capacity) and Gile (1938) grew millet in sand with 15%-17% moisture. Trumble and Strong (1937) and Trumble and Shapter (1937) maintained clovers and grasses in sand culture at 60%-70% of the water saturation capacity by frequent weighing.

Free drainage is now regarded as essential for healthy growth in sand culture. It may be noted, however, that Gile and Fenstel (1943) found that millet grew equally well in drained or undrained sand cultures when kept at 15% moisture, but that the plants grew still better in drained cultures maintained at 27% moisture. No data were given for the undrained ones at this moisture level.

Hicks and Tincker (1944) spread a layer of fine sand over the surface of coarser sand to reduce evaporation from large beds. Trumble and Strong (1937) placed a layer of gravel over sand for the same purpose. Fine sand, however, is more susceptible to algal growth and this may be reduced by a layer of coarse quartz particles or glass beads, as shown by Reed and Haas (1923 a), Mehrlich (1935), Eaton (1936), Gauch and Wadleigh (1943) and Hayward *et al.* (1946). The algal growth is checked by the intermittent drying and wetting.

Mitchell (1939) grew pine trees in pot sand cultures in which the lower five inches consisted of coarse sharp sand and the upper three inches of a mixture of two parts coarse and three parts fine sand in order to obtain free drainage ; two layers of different grades of sand were also used by Meyer (1945) (1946).

Chemical Analysis and Purity of Sand

Macronutrient Constituents

Table 7 shows results of chemical analyses of several sands mostly used in culture experiments. The determinations are of water soluble or acid soluble fractions and do not include the occluded fraction which is probably not available to plants. Samples of sand available at Long Ashton were extracted continuously with redistilled 20% hydrochloric acid in a Soxhlet apparatus until the supernatant acid was colourless for two cycles. The data in Table 7 show that the macronutrient constituents of silica sand are low, but not necessarily negligible as shown by the work of Trumble and Strong (1937) and Trumble and Shapter (1937) on nitrogen nutrition of mixed cultures. It has also been shown by Wallace (1930), Hewitt (1946 a), (1947 a), (1949 b) that the calcareous fraction may seriously affect the precision of experiments on calcium deficiency and the relationship of calcium compounds to other elements, particularly the toxicity of aluminium. van Schreven (1934 b) also found that there was sufficient calcium in sand to delay the onset of calcium deficiency in potato. Cibes *et al.* (1947) failed to obtain calcium deficiency in vanilla grown in gravel owing to the presence of a few calcareous pebbles.

Micronutrient Constituents

The micronutrient content of sand is relatively more serious and the content of all micronutrients, although small, is usually too high for satisfactory use in deficiency studies. The principal impurity is usually iron (together with aluminium). The iron content of many sands is readily available and adequate for the needs of several plants. The iron-bearing fractions in sand may occur in several forms as follows : a brittle concretion heavily contaminated with iron ; coarse particles of mineral ferric oxide ; fine iron-bearing clay and silt ; finely divided mineral particles passing a 100-mesh sieve may also occur.* Iron compounds are also adsorbed as a stain on the surface of the grains, and occluded or dissolved in the grains. Microscopic examination of sand grains stained with potassium ferrocyanide and hydrochloric acid shows that the grains are often irregular, pitted and have surface cracks. Much of the iron containing material appears to be located in these minute pits and along the cracks.

Manganese and boron appear to occur next in order of abundance and may also be sufficient for plant needs. Colwell (1943), determined the available boron in sand by a biological assay with sunflower. The result represented about one-tenth of the total acid soluble fraction found in *other* sands by the writer. Löhnis (1937) found that tourmaline, which is a common constituent of sand, was available to runner bean and tomato plants. van Schreven (1934 a) found that untreated sand contained appreciable amounts of available boron for tobacco. Titanium and vanadium may also be present in considerable amounts according to Hobbs (1944) and Jensen and Betty (1943). The levels of copper and zinc are quite significant and the amount of molybdenum is probably adequate for many plants. The available molybdenum determined by assay with *A. niger* (Table 18) is about ·001 p.p.m. and the acid soluble fraction ranges from ·005 to ·03 p.p.m. according to sample and origin. The impurities introduced into a culture solution percolating through sand may include relatively large amounts of manganese, copper, zinc, lead and other heavy metals as found by Guest and Chapman (1944). Bronsart (1949) leached Hohenbockaer sand with water, citric and hydrochloric acids and detected appreciable quantities of several metal elements including boron, aluminium, iron, manganese, chromium, nickel, zinc, vanadium, silver, lead and barium in each fraction.

It may be fallacious to conclude that glass is a purer form of aggregate than the sand from which it is made.

* Private communication from Dr. Sherwood, Chief Chemist of Messrs. Pilkington Bros., St. Helens, Lancs.

Relation to Grading

The micronutrient and some other impurities in sand may be more abundant in some fractions of sand than others. Thus the fragments of iron sandstone and small calcareous concretions were collected and analysed separately from the bulk of the sand (Hewitt unpublished), and found to contain high concentrations of several micronutrient elements in addition to iron (Table 7). These results have been applied in the development of the procedure for purifying sand used for micronutrient deficiency experiments by the inclusion of a preliminary sifting to remove the coarser particles.

Oertel and Prescott (1944) found that the iron-bearing fractions of ironstone gravels were associated with high concentrations of vanadium, molybdenum, gallium, zinc and lead, but not cobalt, copper, manganese or nickel. Guest and Chapman (1944) also indicated that magnetite, a natural iron mineral, may be associated with injurious amounts of copper, zinc and also cobalt, nickel and chromium.

Toxic Constituents

The micronutrient impurities in sand may not only be sufficient for growth requirements but in less pure sands some, in particular copper and zinc, may cause toxic effects, especially at a low nutrient pH when their solubility is increased, as found by Guest and Chapman (1944). For this reason the sand used should always be a pure silica or quartz sand, and not less than 99·7% SiO_2. Inland white sands are preferable to coloured sands and are found in Europe, in N.W. Scotland, Belgium, Norway and Hohenbockaer in Silesia, as well as in several areas of America. The sand obtained from Leighton Buzzard in Bedfordshire is a pale iron-stained sand and is non-toxic at all pH values used. Ayres (1917) used a pale beach sand, Eaton (1936) used Florida beach sand, and Kenworthy (private communication) a dune sand containing only small concentrations of macronutrient compounds.

Sand Purification

Use of Unpurified Sand

In spite of the frequent occurrence of significant amounts of available micronutrients in sand some investigators have found that culture sands may contain insufficient available micronutrient supplies for normal growth and may even be suitable for use in deficiency experiments without special treatment. McMurtrey (1929), Muckenhirn (1936) and Chandler (1941) found that high-grade silica sands or leached river sands were satisfactory for the production of boron deficiency without further treatment, although that used by Muckenhirn (1936) held considerable manganese. Miller (1928) was able to observe manganese deficiency in several crops grown in untreated sand. Chapman (1939) found that sand did not supply sufficient iron to citrus grown in drip-percolated cultures, but that there was sufficient available iron if the cultures were watered intermittently. Shive (1938), detected no difference in the incidence of boron deficiency between plants grown in water-washed and cold 5% hydrochloric acid—leached sand when using a flowing drip culture. Boron deficiency was induced in several crop plants in 2-3 weeks ; symptoms of manganese deficiency were also produced without treatment of the sand. Alben *et al.* (1942) readily produced boron and calcium deficiencies in pecan before even those of other macronutrients in " washed " glass sand.

Methods and Results using Purified Sand

The data and results reviewed show that sands vary greatly as regards the availability of micronutrients, and consistently satisfactory results cannot be expected with micronutrient deficiencies, unless the sand is treated to remove the available amounts present. A similar conclusion holds for calcium deficiency and probably for precise experiments on the supply of other macroelements. Many workers have stated that " purified sand was used . . ." without specifying the details.

PLATE D

Sand treatment room. Arrangement of sand purification equipment, sheet rubber draught screens and fan.

[*To face page* 31

The usual treatments are water leaching, dilute acid leaching, and treatment with hot concentrated acid. The use of hot concentrated acid followed by calcining as a method for sand purification was described by Salm-Horstmar as early as 1851, and he was able to produce symptoms of iron and of manganese deficiency in oat grown in sand thus treated. McHargue (1923 b) and Hageman *et al.* (1942) used sand treated in small batches with hot 1 : 1 nitric-hydrochloric acids for the production of manganese deficiency and similar sand was used by McHargue and Calfee (1932 a) for boron deficiency studies. Zinzadze (1926) used sand treated with concentrated hydrochloric acid in experiments to test the relative value of different nutrient solutions. Bishop (1928) produced manganese deficiency in several plants grown in beach sand that was first boiled in water, then treated with concentrated hydrochloric acid, 30% nitric acid and finally leached until free from Fe, Cl and NO_3 ; the treated sand was white.

Large-scale methods for purification of sand with hot mineral acids have not been widely attempted. Hewitt (1946 a) described a method for treatment of sand in batches of 40-60 lbs. This was extended and improved to deal with 250 lb. batches in an automatically-operated unit. The procedure is given in detail in Part Two. The acid mixture consists of 15%-18% hydrochloric acid (pure) and 1% oxalic acid. The oxalic acid is included because Adams (1935) and Curtin and Parker (1940) found that ferric iron compounds present as stains or relatively insoluble particles are more easily dissolved in the presence of this reagent. Steam is used to heat and circulate the sand as described in Part Two. Sand thus treated is satisfactory for the study of deficiencies of iron, manganese, copper, zinc, boron and molybdenum. Arndt and Hoffman (1937) were unable to remove all the copper present in Hohenbockaer glass sand by use of hot 10% hydrochloric acid and only produced mild symptoms of copper deficiency in oat as compared with a water culture technique.

Leaching by cold solutions of mineral acids has been used on several occasions. Pfeffer (1918) describes the methods used by Helriegel in 1898 and by Mayer in 1901. The former treated batches of 220 lb. (100 kg.) by allowing it to stand saturated with 33 kilo of concentrated hydrochloric acid for five days in a large clay pot. The sand was then flooded for seven hours with running tap water before being transferred to glass pots and leached with distilled water until free from chloride. The sand was finally calcined to remove the last traces of acid. Mayer leached sand with nitric acid before making a study of the effect of chlorides on plant growth. Hoagland (1919) used sand leached with hydrochloric acid and tap water in experiments on the comparison of sand and water cultures. Hayward *et al.* (1946) used 0·3% of magnetite in 12-ton batches of sand *before* leaching first with water, then dilute nitric acid and finally with water. van Schreven (1934 b) compared untreated sand, sand leached with concentrated hydrochloric acid and a water culture technique for the production of calcium deficiency in potato. The acid-treated sand was greatly superior to the untreated sand and equal in effectiveness to the water culture technique. A similar comparison with similar results was obtained with boron deficiency in tobacco and potato by van Schreven (1934 a) (1939). Hewitt (1947 a) found that sand saturated for five days with cold 2% hydrochloric acid before leaching was superior to untreated or water-washed sand for the production of calcium deficiency and this treatment is recommended for all precise work with macronutrient elements. Kalin (1943) used acid-leached sand for work on low levels of phosphorus supply. Weinberger and Cullinan (1936) leached sand with cold 5% hydrochloric acid and then with water until free from chloride for work with peach trees.

Minarik and Shive (1939) and Kolodny and Robbins (1940) leached sand first with 5% hydrochloric acid and then with 5% ammonia solution to remove boron or potassium. Robbins (1946) suggested leaching sand repeatedly with 3% hydrochloric acid followed by 3% sodium hydroxide and finally excess of water and a dilute nutrient or buffer solution. Chandler (1941) also at first used 5% acid, but this was later thought to be unnecessary.

Scholz (1936) leached Hohenbockaer sand with cold 10% hydrochloric acid followed by excess of water until free from chloride. Flax was grown in the purified and unpurified sand

with and without the addition of micronutrients and the following yields (dry wt.g) were obtained :—

Scholz (1936)

Unpurified sand, no micronutrients added ...	1·97 g.
Purified sand, no micronutrients added ...	No growth
„ „ + Mn, B, Cu	0·95 g.
„ „ + Mn, B, Cu, Zn	1·65 g.

Treatment of sand with water alone is a satisfactory method of removing finer particles of silt and clay, which may otherwise supply significant amounts of available bases and present an extensive surface for base-exchange reactions.

Upward displacement by a stream of water was recommended by Shive (1918 a), Spencer and Shive (1933) and Robbins (1946). Shive (1920 a) found that water washing was able to remove the absorptive fraction in the sand. Water washing was used for routine experiments with macronutrients by Hewitt (1944-1946). Blake *et al.* (1937) used water-washed silica sand for growing fruit trees. Trumble and Strong (1937) and Trumble and Shapter (1937) used leached river sand thoroughly washed with water continuously for 3-4 weeks in large pots, for work on nitrogen fixation and transfer in mixed legume—non-legume cultures. Arnon and Meagher (1947) compared analyses of leachates of high grade silica and Ottawa sands using water (pH 7·4) or dilute hydrochloric acid (pH 2·3) or equivalent sodium chloride (pH 7·1). Appreciable amounts of magnesium, potassium and particularly calcium were extracted and the calcium fraction was markedly increased in the acid leachate. The results obtained with the sodium chloride solution indicated also that there was a significant amount of calcium in exchangeable form that was not soluble in water but was less than the total extracted by the acid.

Sand that has been treated with acid may be highly adsorptive, as noted by McHargue (1923 b) and Hewitt (1947 a), and is saturated with hydrogen ions. This means that leaching with water only after treatment may not be effective in removing the absorbed hydrogen ions which are, however, exchanged for basic ions such as calcium, magnesium or potassium present in a nutrient solution. Contact between sand, that has been leached with water only until free from chloride or free acid, and a neutral nutrient solution was found by the writer to result in a rapid drop in pH and an injurious concentration of hydrogen ions was produced. For this reason it is necessary to leach acid-treated sand with an appropriate nutrient solution until there is no change in pH on standing for some hours, as described in Part Two. This liberation of exchangeable hydrogen ions occurs in sand that has been treated with hot or cold acids, and may even be greater from the latter. The observations of Zinzadze (1926) who found that the osmotic pressures of several culture solutions were greatly increased when in contact with acid-treated sand may have been due to this effect. His sand cultures were also frequently unfavourable in comparison with water cultures, probably owing to the injurious concentrations of hydrogen ions liberated. Shive (1920 a) observed that untreated sand did not influence the pH or concentration of solution brought into contact with it unless very fine particles were present, when their adsorptive capacity reduced the concentration of a solution by as much as 8%. The adsorptive capacity of acid-treated sand was sufficient to cause error in some experiments of Arnon and Grossenbacher (1947) and Arnon and Meagher (1947), where base exchange materials were admixed with the sand.

Mixed Solid Media

Many experiments have been made in which sand has been mixed with other solid material. Early experiments of this type have been reviewed by Pfeffer (1918), pp. 80-97.

These methods comprise two main objectives : (1) the incorporation of base exchange materials such as bentonite or other clays, ion exchange resins or zeolites to modify the availability or combination of nutrients ; (2) the addition of solid nutrient salts or the use of particular compounds such as magnetite, calcium carbonate or relatively insoluble phosphates to supply a particular element in solid form. Work of this type is particularly suited to sand

TABLE 7

Data on Mineral Impurities in Silica Sands

Reference	Sand	Fraction and Extraction Method	SiO_2%	N	P_2O_5	CaO	MgO	K_2O	Fe	Al	Mn	Cu	Zn	B	Mo	V	Ti, etc.
			Values as parts per million														
Arndt and Hoffman (1937)	Hohenbockaer Glass Sand	Water Soluble										·0042					
,, ,,	Ditto	Total										·09					
Arnon and Meagher (1947)	Silica	Water Soluble (pH 7·4) ...		·5	·15	61	7	9·5									
,, ,,		Exchangeable (0·01 M. NaCl pH 7·1)		5	·15	96	7	9·5									
,, ,,		Acid Soluble (0·01 M. HCl pH 2·3) ...		5	7·5	130	13	13									
,, ,,	Pure Ottawa Sand ...	,,				20	4 (approx.)										
Ayres (1917)	Pale Beach Sand ...				40	600	2,800	Trace	4,000	2,500							
Colwell (1943)		Biological assay : Sunflower												·02-·05			
Davis (1927)...	Silica		98·84		500	1,000	400	700	4,500 (as $Al_2O_3 + Fe_2O_3$)								
Hayward, Long and Uvits (1946)			94·0					20,000	15,000 (as Al_2O_3)								
Hellriegel (1898)	Hohenbockaer Glass Sand	Cold HCl	99·67	4-50	Trace	80	30	24 (4·5 Na)									
Hobbs (1944)	Ottawa Silica Sand ...	HCl	99·77			Nil	Trace		180	800							TiO_2 140
Hughes (1943)	Seashore Silica Sand ...	Total Spectrographic ...				* †	†		†		†	†		*N.D.	N.D.		Ti †, Co, Ni ; N.D.
Jensen and Betty (1943) ...														·0046	2·5-10		
Muckenhirn (1936)	High Grade Silica Sand	Cold H_2SO_4									20		N.D.				
Scharrer and Schrop (1933)	Hohenbockaer Glass Sand	HCl		70	20	190	200	150									
Trumble and Strong (1937) Trumble and Shapter (1937)	Leached River Sand ...			43	70-90												
Waltman (1931)	Fine White Silica Sand		99·55						470	1,050							
Weinberger and Cullinan (1936)			99·71			120	60		346								
Kenworthy (private communication)	Dune Sand (Inland) ...	Soil extraction method ...			14	50	4	6·3	8·4		·7						

* † = Present. N.D. = Not Detected.

[*To face page* 33

culture methods and finds special application in the study of base exchange in relation to availability and antagonism and the uptake of elements from relatively insoluble compounds. These and other purposes are considered below. The use of magnetite is discussed in detail in another section on the supply of iron to culture plants.

TABLE 7—*continued.*

Data on Mineral Impurities in Silica Sands

Reference	Sand	Fraction	Fe	Mn	Cu	Zn	B	Mo	Mo‡
			Values as parts per million						
Hewitt (mainly unpublished data) All samples extracted by boiling 20% HCl	Leighton Buzzard, Double Arches Pits* ... 1943	Unsieved ...	360	·28	—	—	3·5	—	—
	Ditto ... 1944	Sieved through 24-mesh, copper gauze	240	·25	—	—	·6	—	—
	Ditto ... 1945		170	·15	—	—	·7	—	—
	Ditto ... 1946		150	·29	·2	—	·4	·010	—
	Ditto ... 1948	Retained 24-mesh ...	212	·8	·7	2·7	2·25	—	—
		Passed 24-mesh ...	142	·15	·7	1·6	1·55	—	—
	Ditto ... 1949	Retained 24-mesh ...	463	·6	2·3	—	1·3	·012	·00135
		Passed 24-mesh ...	196	·3	1·0	2·1	4·0	·032	·00285
	Fragments of iron sandstone and calcareous concretions on 24-mesh sieve		72,000	295	30	—	33	·25	—
	Heavily iron-stained sample		3,802	16	1·9	13·5	3·7	·015	·0028
	Pink stained profile ...		488	·76	·39	·82	4·0	·017	·0027
	Exface 1949 (Front Pit)		136	·1	·27	1·75	1·0	N.D.	·00065
	Exface 1949 (Big Pit) ...		257	·4	·25	·55	4·7	·006	·0009
	Average values (Big Pit) assessed 1943-50 (as used)		216	·26	·55	1·4	2·2	·018	—
	Aylesbury Pit "60" ...		296	1·3	·16	2·25	3·1	·047	·00215
	„ „	carbonate boiled	320	10·7	·07	0·7	1·0	·051	·0036
	Loch Aline (N.W. Scotland)	White sand retained by 1 mm. (24-mesh)	13,410	317	7·8	—	12·5	·32	—
	„ „	Passed by 1 mm. (24-mesh)	125	1·6	·9	3·7	·60	·015	·0035
	Leziate Sands:								
	Washed No. 1 ...		130	·3	·4	1·0	1·4	·043	·0012
	Fine		222	·6	·6	N.D.†	2·9	·019	·0011
	Coarse		212	·3	·35	2·5	3·1	·044	·0011
	SiO_2 99·8%								

*{ SiO_2 99·8%; Ca 40 p.p.m. approx.

†N.D. Not Detected.

‡Available Mo determined by *A. niger* (Hewitt and Hallas 1951).

Base-Exchange Materials

The advantages claimed for the use of ion-exchange materials to supply some or all of the macronutrient elements include the following : The ratio or concentration of cations or of anions supplied in adsorbed form may be varied almost independently of each other, since the common "ion" is represented by the insoluble exchange medium and the accompanying ion of opposite sign for each metal or acid ion given in solution is eliminated. The effects of osmotic pressure due to varied concentrations are minimised and so are the variations in pH due to differential uptake. The resistance to leaching of adsorbed nutrients makes them useful for work in outdoor cultures. Undesirable effects due to precipitation of nutrients, e.g., calcium and iron compounds, may be avoided. These adsorption materials present a promising field for the study of effects of the ratio of various cations, including the hydrogen ion, on the *availability* of a particular cation, e.g., Ca^{++} supplied at different concentrations by weight and different percentages of saturation. These studies have an important bearing on the understanding of physico-chemical relationships of the availability of nutrients in soils and have been investigated in detail mainly by Albrecht, by Arnon *et al.* and by Jenny *et al.* as stated below.

Certain difficulties attach to the use of base-exchange materials in sand culture. Mistakes in the mixture or preparation of the adsorption complex may be costly and cannot easily be rectified, and changes cannot be made during an experiment. The organic resin type of ion-exchange materials may be decomposed by bacterial attack and the introduction of organic material may involve other difficulties. It cannot always be assumed that preparation of a particular mixture of ions in adsorbed form will remain in equilibrium when brought into contact with water, let alone other ions in solution. It may not be possible to supply nutrients in the same ratios as in culture solutions.

Materials commonly used comprise three types, namely, synthetic resin materials, zeolites and clays ; some examples are given in Table 8, together with data to show their relative adsorptive capacity.

It will be seen from Table 8 that the zeolites and synthetic resins have greater exchange capacities than the clays. The exchange capacities of the resins shown are only approximate and vary with the particular ions. The capacity is considerably reduced for weakly dissociated ions. Thus data given by Arnon and Grossenbacher (1947) for Amberlite resins show that the relative saturation values for SO_4'' and H_2BO_3' on IR 4 are nearly 14 : 1 and the relative values for Ca^{++} and Mn^{++} on IR 100 are about 1·6 : 1. Divalent (and trivalent) ions are also adsorbed more strongly—i.e. the resins exhibit a greater capacity for these—than monovalent ions. Partially dissociated polyvalent anions such as the phosphate anion may not be completely adsorbed. Thus, whilst the theoretical capacity of IR 4 for PO_4''' is 1368 mg.eq./100 g., the true value is only 456 mg.eq./100 g. as H_2PO_4', Arnon and Grossenbacher (1947).

The resins and zeolites are granular and are easy to handle and mix into sand or other aggregate. They can be saturated either by shaking in a solution of the appropriate ion or by continued perfusion in a column. For total adsorption of a single ion the latter method is preferable as the reaction is more nearly complete when it is carried out on a chromatogram. Although the resins or zeolites are normally mixed in sand and comprise, usually, about 0·2 to 4·5% of the weight of the medium, they were used undiluted by Schlenker (1940) in an experiment with maize.

The clays cannot be readily perfused and are usually handled by shaking a suspension of the electrodialysed clay with a solution of the appropriate ions or by titrating the hydrogen-saturated clay until the required degree of exchange has been produced. The clays are not so convenient a rooting medium as the resins. They are usually mixed with sand, fine gravel or vermiculite at concentrations between 1·0 and 7·0% by weight, the higher concentration being necessary to balance lower capacity. The fine particles probably cover the sand or other aggregate and present large surface areas, without impairing the aeration and capillary properties of the medium.

TABLE 8

Types and Exchange Capacity of Some Base-Exchange Materials

Base-Exchange Material	Type	Properties	Exchange Capacity (mg.eq./100 gm)
*Amberlite IR 100	Synthetic Resin	Cation Exchange ...	120
,, ...	(Phenol formaldehyde type)	,,	
*Amberlite IR 4	Synthetic Resin	Anion Exchange ...	220-280
,, ...	(Amine formaldehyde type)	,,	
†Permutit Zeo Carb H ...	Sulphonated Coal	Cation Exchange ...	200
Zeo Carb 215 ...		,,	
†Permutit DeAcidite B and E	Aniline Polymer	Anion Exchange (General)	120
,, F ...		,, (for *weak* acid anions)	
,, Biodeminrolit ...		Mixed Bed Exchange of high efficiency	
Dusarit		Cation Exchange ...	200 at 90% saturation
Aniline Black		Anion Exchange ...	122 for SO_4'', NO_3', 66 for PO_4'''
Decalso	Artificial Sodium Zeolite ...	Cation Exchange ...	230
Permutit	Natural Zeolite	,, ...	
Bentonite	Otaylite clay (Arnon and Meagher 1947)	,, ...	120
Bentonite	Montmorillonite clay (Converse *et al.* 1943)	Cation and Anion Exchange	80
Electrodialysed clay	Colloidal clays (Horner 1936)	Cation Exchange ...	66 (97% saturation)
Miami Sub-soil clay Colloid ...	(Converse *et al.* 1943) ...	,, ...	65
Putnam Silt-loam clay Sub-soil	(Wittwer *et al.* 1947) ...	,, ...	28

* The Resinous Products Company, Philadelphia, U.S.A.
† The Permutit Company, Chiswick, London, W.4.

Albrecht (1946) gave a detailed description of a simple electro dialysis apparatus for the preparation of colloidal clays. This produced a continuous supply of 500 g. dialysed clay weekly. Full details of the incorporation of nutrients in the clay and its admixture in the cultures were also given.

The earliest experiments on the growth of plants with base-exchange materials appear to have been made with zeolites, by Nostitz (1925) and Breazeale (1928). Ca^{++}, Mg^{++}, K^+ together with Na^+ and N as NH_4^+ were given to oat and barley in absorbed form on Permutit zeolites at the rate of 0·2-0·5% by weight in sand in early experiments by Nostitz (1925). Phosphorus was given as solid aluminium phosphate. The results were superior to those obtained when all the salts were presented at the same concentration in solution, but the best results were obtained when potassium was given as the chloride in solution while the calcium and magnesium were given in the adsorbed form. This observation was clearly confirmed by the work of Arnon and Grossenbacher (1947) discussed below.

(a) *Zeolites and synthetic resins.* Schlenker (1940) used "Decalso" zeolite to grow maize with Ca^{++}, Mg^{++} and K^{+} ions. NO_3', PO_4 (probably as H_2PO_4') and SO_4'' ions were given on aniline black. Micronutrients were given according to Hoagland A—Z formula (Hoagland and Snyder (1933)), and iron was supplied weekly as ferric citrate solution. The adsorption materials were mixed to give the following milligram-equivalent amounts of the various ions :—Ca^{++} 18·4, Mg^{++} 5·5, K^{+} 17·2, NO_3' 22·0, PO_4''' 1·6, SO_4'' 8·8, which was regarded as the "standard" nutrient. It may be noted that these values are greater than those now normally given in a solution and that the sum of nutritional anions and cations is not equal. The maize plants were grown in water culture (not sand culture) in contact with the ion exchange materials and the cultures were aerated. The "standard" nutrient was compared with similar cultures containing $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$ of the total exchange mixture and with Shive's R_5S_2 containing 13 mg./eq. KH_2PO_4, 10·4 mg./eq. $Ca(NO_3)_2$ and 30 mg./eq. $MgSO_4$ changed weekly and unchanged. The standard and $\frac{1}{2}$ standard adsorption types gave similar growth. The percentage yield for the remaining dilutions were 82, 78, 50 and 45 respectively. The R_5S_2 changed weekly gave 50% and the unchanged 45% yield respectively. The ion exchange types thus provided a more suitable nutrient medium than the Shive's solution. They also supplied a greater reserve of nutrient ions than many nutrient solutions and the results were superior to those obtained when high concentrations were given in a single batch of nutrient solution. Schlenker (1942) extended the method to soya bean and kidney bean. In this work similar ion-exchange materials were mixed with quartz sand, which was saturated with water in a container where the drainage was restricted by the level of a glass outlet tube so that some water remained in the base of the pot. The milligram-equivalent concentrations were Ca^{++} 3·46, Mg^{++} 1·60, K^{+} 0·80, NO_3' 3·46, HPO_4'' 2·4, SO_4'' 1·6 and the anion and cation levels were balanced. These cultures were compared with ordinary sand cultures and found to be superior to them.

Lehr (1942) grew sugar beet in a 4% Dusarit and sand mixture. The ions Ca^{++}, Mg^{++}, K^{+} and Na^{+} were given in the ratio of 78 : 6 : 8 : 8, but 10% of the total exchange capacity was taken up by H^{+} ions. The Dusarit preparations were made by percolating the acetate salts of the metal ions separately and mixing afterwards in the desired proportions. Phosphate as $Ca(H_2PO_4)_2$, nitrogen as $Ca(NO_3)_2$ or $Na(NO_3)$, iron as $FeCl_3$ and micronutrient elements were all given as solid salts. Bower and Wadleigh (1948) found that a Ca : Mg : K ratio of 10 : 3 : 1 gave best results with Dallis grass, when amberlike resins were used to provide 8 and 4 mg.eq. cations and anions respectively per 100 g. sand/resin mixture.

Converse *et al.* (1943) compared the effects of using clays, artificial zeolites and ion-exchange synthetic resins as sources of adsorbed nutrients for maize, grown in irrigated sand cultures. Iron and boron were supplied as bentonite adsorbates with or without the inclusion of phosphate in the same form. The experiments included the study of deficiencies of N, P, S, Ca, Mg, K and B. The adsorbents were mixed with the sand with the aid of a rotating barrel provided with a diagonal baffle.

Graham and Albrecht (1943) found that maize contained a greater total nitrogen percentage when supplied with calcium nitrate in solution to a N-deficient sand-soil mixture than when the medium was mixed with an Amberlite resin containing adsorbed NO_3' ions. The nitrate was, however, less readily leached from the Amberlite mixture than from the medium given nitrate in solution. Peterburgsky (1945) studied the uptake of Ca^{++}, Mg^{++} and K^{+} ions adsorbed as Permutit zeolites mixed in sand and from solution given to sand only. Less total amounts of these ions were absorbed from the zeolite than from solution by barley and oat, but the dry weight production was greater. It was concluded that uptake occurred directly from the solid (adsorbed) phase.

Arnon and Grossenbacher (1947) and Arnon and Meagher (1947) made a detailed study of the effects of using nutrient ions adsorbed on Amberlite synthetic resins and their observations and conclusions merit considerable attention. The object of this work was the production of fresh vegetables by members of the U.S. Army Air Force where production in soil was not possible. The use of exchange materials was examined as it was thought to be a means to simplify the manipulation of large-scale sand or gravel cultures used to grow vegetables.

In order that only water should have to be applied an attempt was made to supply all the micronutrient elements also in the adsorbed state (except for copper, zinc and molybdenum for which natural impurities were relied on to supply sufficient amount). Tomato plants of several varieties and lettuce were used and grown in sand or in solution only, both in large (460-litre) tanks, 8 inches deep and in individual, automatically-irrigated pot cultures described in another section. Amberlite resins IR 100 and IR 4 previously saturated with the requisite nutrient ions were mixed in the sand in the following proportions :—

Arnon and Grossenbacher (1947)

Ion	mg./eq. per tank	Wt. of resin gm.
Ca^{++}	8,650	5,865·2
Mg^{++}	3,840	2,660·5
K^{+}	14,200	12,032·3
Fe^{++}	34·6	24·2
Mn^{++}	21·4	22·5
NO_3'	21,270	6,387·0
H_2PO_4'	972	258·7
SO_4''	3,880	754·5
H_2BO_3'	31·3	80·0
Wt. of sand 630 Kg.	Total	28,084·9
% by Wt. in sand 2·25		

These amounts were arranged to correspond with the total absorption of nutrients determined in earlier experiments by Arnon and Hoagland (1940) with tomatoes grown for five months. In order to economise in the weight of resins used a duplicate series was tried in which potassium nitrate was given in solution at watering instead of as the adsorbed ions, and this reduced the weight of resins by about 45%. The solution used in the control sand and water culture series had the following composition : Ca^{++} 6, Mg^{++} 4, K^{+} 10, NH_4^{+} 2, NO_3' 16, H_2PO_4' 2, SO_4'' 4 mg.eq./L. Micronutrient elements were given according to the A5 group devised by Arnon (1940).

The tanks were arranged with a slope of about one inch in 10 feet. The sand culture and Amberlite-sand tanks were watered daily with nutrient solution or water only or dilute potassium nitrate solution and the excess liquid was allowed to drain back to the main reservoir after standing in the sand tanks for half an hour.

Six weeks after planting in all cultures, the tomato plants grown in the tank, where all ions were supplied in the adsorbed state, developed typical symptoms of calcium deficiency and made poor growth. These symptoms were not shown in any other tanks, but growth in the tank given potassium nitrate in solution and the other ions as Amberlites was also visibly inferior to the plain sand and water culture series. The calcium deficiency was remedied by application of additional calcium sulphate and also magnesium sulphate in the water for nine weeks. The relative growth is indicated by the following relative data condensed for the three varieties from Arnon and Grossenbacher (1947) :—

Treatment	Shoot Wt. (Three weeks)	Fruit Wt. (Six months)
Sand culture	66·2 gm.	31·2 lbs.
Water culture	103·1	26·6
All nutrients *except* K and NO_3 as Amberlite ions ...	52·3	22·9
All nutrients as Amberlite ions	29·8	14·1

Similar results were obtained from the irrigated pot experiment. Here it was also shown that increase in the *total* amount of Amberlites present produced an even *less* favourable result when all ions were present in the adsorbed state, but a similar increase produced a *more* favourable result when KNO_3 was given separately. This result showed that no toxic

effect of the Amberlites was involved and that the benefit of eliminating the K- and NO_3-Amberlite was not due to reduction in the Amberlite concentration. A marked beneficial effect was also produced when sodium chloride was included in the water given to the " all Amberlite " tank, but the effect was detrimental in the presence of KNO_3 given separately. Slight consistent improvements were only obtained in " all-Amberlite " series when the proportions of Ca-Amberlite were increased 9-fold. None of the Amberlite treatments, however, was as satisfactory as the plain sand culture control series.

It was concluded from these results and chemical analyses of the leachates and foliage and from further detailed investigations by Arnon and Meagher (1947) that when all the nutrient ions were given in adsorbed form the availability of calcium (and also of magnesium) was greatly reduced ; sufficiently so as to induce acute mineral deficiency effects. The elimination of K^+ from the adsorption complex naturally had the effect of increasing the ease of removal of Ca^{++} and Mg^{++}, since it must be concluded that once the separately prepared " Amberlite ions " were intimately mixed in contact with the aqueous phase the whole behaved as though a single batch had been treated with a mixture of the ions in one solution. Under these conditions the true partial percentage saturation for Ca^{++} and Mg^{++} was much less and the ease of desorption was also reduced. When K^+ or Na^+ were added to the Amberlites fully saturated by Ca^{++} and Mg^{++}, these ions were partly exchanged and became more available. As KNO_3 was given at each watering the reaction was always encouraged to proceed and the increased availability was maintained until the equilibrium was attained with the residual adsorbed Ca^{++} and the K^+ in solution. That this state was ultimately reached was shown by the fact that the lettuce grown in the tank given KNO_3 finally developed calcium deficiency and showed a low calcium status. These results probably explain the superiority of the separate addition of potassium as chloride observed by Nostitz (1925). They may also be significant in the response to sodium found when ion exchange cultures or soils were used as culture media (cf. for example Lehr (1941), (1942), (1947)).

Arnon and Grossenbacher (1947) also pointed out that far more NO_3' ions appeared in solution from the adsorbed state than were expected and considered that this might be due partly to the presence also of cation-exchanging Amberlites (which were not included in the work of Graham and Albrecht (1943) referred to above), and also to significant amounts of chloride present initially in the sand. There was also a tendency for the plants to have a low phosphate status and this was attributed to the fact that the capacity for phosphate adsorption had been wrongly calculated as PO_4''' instead of H_2PO_4'. The continued work of Arnon and Meagher (1947) showed that in the presence of the K-Amberlite about eight times the concentration of the adsorbed divalent ions Ca^{++} and Mg^{++} were required to produce the same concentration in a leachate solution as when the K-Amberlite was omitted from the mixture, but the availability of the K^+ was scarcely affected as compared with the K-Amberlite alone. It was also found that sulphate and especially phosphate availability were affected in a similar manner in relation to nitrate, but that increase of these also increased the desorption of nitrate. The difficulties encountered by Arnon and Grossenbacher show that the use of ion-exchange materials may present serious problems when attempts are made to supply all nutrient ions in adsorbed form, but it may be seen from the other more favourable experiments reported here, that the ratio of $Ca^{++} + Mg^{++}/K^+$ has been much higher than the 46·6% of total divalent ions used by Arnon and Grossenbacher, except in some of the irrigated pot experiments.

It is, however, clear that in order to obtain satisfactory results a $Ca^{++} + Mg^{++}/K^+$ ratio considerably in excess of that used in ordinary nutrient solution is necessary when working with some ion-exchange materials.

(b) *Colloidal clays.* The use of natural colloidal clays as base-exchange materials has also received considerable attention. Much of this work has been concerned in the investigation of calcium uptake by Albrecht (1932), Albrecht and McCalla (1938), Horner (1936), Graham (1941), Graham and Albrecht (1943), Mehlich and Colwell (1946) and Wittwer *et al.* (1947). Albrecht and McCalla (1938) found that soya beans were able to utilise calcium in the adsorbed form and that increase in total calcium level and in percentage saturation of the

clay were both beneficial, but that increase in saturation was more beneficial than increase in weight of clay for equivalent amounts of calcium, especially at low saturation levels. Similar results were obtained with magnesium. Horner (1936) found that soya bean made 250 times the growth with a total of 0·3 mg.eq. Ca^{++} as at 0·05 mg.eq. per plant and that the growth when Ca^{++} was given at 97% of the saturation capacity of the clay was twice that at 40% saturation, when the same total amount of Ca^{++} (0·2 mg.eq. per plant) was supplied. Jenny and Cowan (1933) found that soya beans failed to grow in contact with a Ca^{++} 1-1—bentonite system if the Ca^{++} saturation was less than 30%, even when apparently adequate total calcium was supplied. Mehlich and Colwell (1946) studied the availability of calcium in the adsorbed state as bentonite, kaolin and other clay colloids and also concluded that degree of saturation has an important effect on availability independently of the total amount present. Mehlich and Colwell (1946) found that bentonite yielded less calcium than kaolin at the same percentage saturation and that whereas bentonite yielded calcium in proportion to its saturation, kaolin yielded calcium in proportion to the total weight of the clay present.

Kolodny and Robbins (1940) used bentonite to supply potassium to tomato plants in sand culture. From 1-2% by weight of bentonite saturated with 76 mg.eq.K^+/100 g. and the remainder saturated with Ca^{++} was used. Although fair growth was obtained in the early stages the potassium level was inadequate to prevent deficiency later on. It was found that potassium was readily leached from the bentonite complex especially in drip culture, and auto-irrigator clay candles were used to supply solution instead. Tyner (1935) found that K-bentonite was not superior to soluble K^+ ions for buckwheat and clovers in sand culture.

Nitrate was supplied as an adsorbed ion on clay to lettuce by Jenny (1946). Dean and Rubens (1945) used colloidal clay to study phosphate uptakes, but they were unable to produce evidence of contact exchange from a clay suspension in aerated water culture and did not obtain satisfactory growth of barley under these conditions. Greenhill and Page (1933), and Lewis (1936) grew rye-grass in sand cultures to which 6% by weight of bentonite was added to produce a medium with ' additional " soil type " properties.' 4% of calcium carbonate was also included and the mixture was maintained at 13% moisture. The surface was covered with fine sand to avoid caking or cracking and good growth was obtained. Guest and Chapman (1944) grew citrus in percolated sand cultures where a nutrient solution was continually re-circulated, and mixed 1% of bentonite (and also magnetite) in the sand in the hope that the nutrient ions would be partly adsorbed on the clay and that the effects of extremes of high pH on growth (which represented the object of the work) would interfere less with the availability of certain nutrients. In spite of the bentonite, calcium carbonate was precipitated in the medium at the " more alkaline pH values " and slight phosphorus deficiency developed. Growth, however, was good between pH 7—8·8 and fair even at pH 9·7.

The ability of clays to hold both anions and cations simultaneously in adsorbed form is in contrast with the previous group of resins and zeolites considered above. Arnon and Meagher (1947) found that bentonite and Amberlite IR 100 behaved similarly as regards their ability to exchange Ca^{++} and K^+ ions for H^+ ions when the former were adsorbed in various proportions on each of these materials. Graham (1941) found that the calcium as Anorthite was not readily available to soya bean roots in contact with it, but that when a hydrogen-saturated clay was also introduced the plants were able to take up calcium and a transfer mechanism between the Anorthite and the root *via* the clay was postulated. The subjects of depletion and absorption following the contact of roots with soil colloids have been developed especially by Jenny and Overstreet (1938) (1939), Jenny *et al.* (1939), and Jenny (1946). In many of these experiments radio-active isotopes have been usefully applied. It has been found that bentonites not only supply ions in the adsorbed state to plants but that, when saturated with NH_4^+, H^+, Na^+ or Ca^{++}, these clays may cause the depletion of K^+ from the plant roots in contact with an aqueous suspension of the clay. The theory of contact nutrition cannot be discussed in greater detail here and reference should be made to the publications cited.

(c) *Other materials.* Experiments probably related to the effect of base-exchange materials incorporated in sand cultures were made by Gile (1939) and Gile and Fenstel (1943). These workers added from ·04-1% by weight of colloidal and peat fractions, silica gel, sodium silicate, colloidal ferric hydroxide or activated charcoal, to sand cultures used to grow millet, mustard, rice and wheat supplied with a complete nutrient solution. Visible beneficial effects compared with control cultures were seen in 4 days and were found to be independent of the supply of moisture or micronutrients. The effects were ascribed partly to elimination of acute pH gradients close to the roots and partly to improved availability of iron. Earlier work on the addition of colloidal materials is reviewed in these papers. Lemmerman and Weissman (1922) and Germar (1934) reported beneficial effects for several crops from the inclusion of colloidal silica gel in sand cultures whilst little or no effect was produced in the presence of mineral silica compounds. Prianisnikow and Domontovitch (1926) obtained improved growth with several crops in the presence of ammonium nitrate, when charcoal was mixed with the sand, and change in pH was reduced. More recently Vantsis and Bond (1950) have re-investigated the effects of activated charcoal added at the rate of 0·5-2·0% by weight to undrained sand cultures. Markedly beneficial results were obtained with peas with increased dry weight and additional nitrogen fixation. The results were attributed to pH stabilisation and removal of toxic root secretions by adsorption. Numerous other experiments were reviewed.

Solid Nutrient Compounds.

Several experiments with various objectives have been made in which some or all of the nutrients or other reactive compounds have been given in solid form to sand or other aggregate. These experiments are reviewed here, except where they relate to the supply of iron. This subject is treated below.

In many of the earlier sand culture experiments, some or all of the nutrients were supplied in solid form mixed with the sand in a single application at the beginning of the experiment. This procedure was used by Ayres (1917), and later by Lemmerman and Weissman (1922) for oat, barley, rye grass, clover and other plants, which were given 2·0 g. N at intervals and 1·0 g. $MgSO_4$ $7H_2O$, 0·25 g. NaCl, 1·5 g. $CaCO_3$, 2·0 g. K_2O as K_2SO_4, 0·2 g. $FeSO_4$ $7H_2O$, and by Gregory and Richards (1929), Richards (1932) and Richards and Templeman (1936) who worked with barley. The weights given were $NaNO_3$ 9·16 g., Na_2HPO_4 $12H_2O$ 2·52 g., K_2SO_4 1·85 g., $CaCl_2$ $6H_2O$ 0·37 g., $MgSO_4$ $7H_2O$ 1·25 g. in 30 lb. of sand. This procedure was slightly modified by Gregory and Crowther (1928) when the total nutrient supply was given in solution to the sand at the beginning of the experiment and by Richards (1938) who added the total nutrients in three batches at germination and two and four weeks later. Hicks and Tincker (1944) grew carnations in large beds where the nutrients were given in solid form as a top dressing at the rate of 1 ounce per square yard per month. It was found that magnesium compounds accumulated rapidly to the extent of 0·05% weight of the sand, but tomato fruit and carnation flowers cropped more heavily than from soil. Trumble and Shapter (1937) supplied nutrients to mixed cultures of legumes and grasses grown in sand culture by a combination of a single application in solution with top dressings of solid nutrients containing nitrogen. van Schreven (1934 b) grew potatoes in normal and calcium-deficient sand cultures where all the nutrients were added in solid form at the commencement.

The technique of giving the essential nutrients in solid form has certain disadvantages. Thus the initial concentration of salts may be far too great and may cause injury by direct contact with roots or by osmotic effects, and in the later stages of the experiments the final concentration may be too low and deficiency effects may occur. Moreover, the presence of all the nutrients in solid form but of varying solubility, availability and rate of uptake is likely to lead to unbalanced conditions of nutrient supply. The method, however, has special applications : (a) when it is desired either to study the availability of certain sparingly soluble or possibly easily revertible forms of particular compounds, (b) where a low solubility combined with fine particle size (as with magnetite or certain precipitated carbonates or phosphates) allows of the supply of a particular nutrient partly independent of antagonistic or other

concentration effects, and (c) where it is desired to study effects of, say, calcium carbonate on iron or boron availability.

van Schreven (1934 b) found that calcium as solid $CaSO_4 2H_2O$ at the rate of 1·5-3·0 g. in 7 kg. sand was adequately available, but when given as $Ca(H_2PO_4)_2$ at the rate of 1·22 g. the tubers showed medullary necrosis, although the foliage was satisfactory. Hewitt (1947 b), (1948 a) found that 10 g. $CaCO_3$ in 9 kg. acid-leached sand was a satisfactory source of calcium for barley, oat, kale, cauliflower, potato, during several months' growth and also for sugar beet during the early part of the experiments, but not in the later stages when mild calcium deficiency developed, or when ammonium nitrogen was given.

Admixture of solid calcium carbonate in sand has been tested in relation to its effect on the availability of iron to plants. Chapman (1939) found that a mixture of 2·5% calcium carbonate and 0·1% magnetite in sand resulted in chlorosis in citrus, but with 0·5% magnetite no chlorosis occurred. The mixture had a pH of 8·2 when continuously perfused with nutrient, but the pH fell to 7·0 when perfusion was stopped, due apparently to the effects of carbon dioxide evolved from the roots. Guest (1944) found that chlorosis in citrus was induced by 0·1% magnesium carbonate or 0·1% tricalcium phosphate in sand cultures containing 0·5% magnetite, whereas 0·5% calcium carbonate did not cause chlorosis. Hewitt (unpublished) observed chlorosis in tomato plants grown in unpurified sand with 0·25% magnesium carbonate but not with 0·5% or 5% calcium carbonate. The pH of the mixture was 7·5 with the calcium carbonate and 8·8 with the magnesium carbonate. Jones (1948 unpublished) found that 0·5% calcium carbonate in sand induced chlorosis in potatoes given a normally adequate level of iron as ferric citrate. The availability of phosphate is also affected by calcium carbonate in sand (Hewitt unpublished). Hewitt (1948 a) found that the toxic effect of aluminium compounds was greatly reduced by quite low levels of calcium carbonate mixed in the sand, as opposed to the much smaller effect of calcium compounds in solution. Hewitt (unpublished) found that toxic effects of ammonium salts were eliminated or greatly reduced by the incorporation of 30-50 g. calcium carbonate in 10 kg. sand. Ferguson and Wright (1940) and Hewitt (1945) were unable to induce boron deficiency (or to accentuate it) by admixture of calcium carbonate in sand.

Several workers have used relatively insoluble phosphates to supply phosphorus in sand cultures. Baguley (1912) compared the availability of aluminium and tricalcium phosphates for swede and oat plants in sand culture. Swedes were well able to absorb phosphorus from aluminium phosphate (a finding that appears to be confirmed by the present writer's work). Nostitz (1925) used aluminium phosphate to supply phosphorus to oat and barley in sand cultures. Tyner (1935) grew several plants in drip sand cultures in which iron and phosphorus were supplied as equivalent amounts of solid ferric phosphate and tricalcium phosphate at a concentration of 0·007% by weight, or as ferric phosphate only at 0·025% by weight. McGeorge and Breazeale (1932) found that freshly precipitated phosphates of iron, aluminium and calcium were readily available, but the natural (mineral) forms of these compounds were much less available, although availability was enhanced by low pH values of 3-4·5. Marais (1922) compared the availability of these compounds in sand cultures.

McHargue and Calfee (1933), and Muckenhirn (1936) used ground pyrex glass as a source of boron in sand culture. Salm-Horstmar (1851) used solid manganese carbonate to eliminate symptoms in oat (now known as " grey speck " disease due to manganese deficiency) that occurred when excess of solid ferric oxide, carbonate, or phosphate was given.

The use of solid, sparingly soluble compounds provides a relatively constant supply of some elements. The low solubility may reduce the risk of toxicity, but this is not invariably so, as shown by the work of Guest and Chapman (1944) on magnetite (q.v.), and the relatively constant " active mass " provided may eliminate or modify many problems of antagonism in nutrient uptake. With the exception, however, of the special objectives considered, the use of solid nutrients does not (in the opinion of the writer) supersede the more orthodox procedures in which nutrients are supplied regularly at low concentrations in soluble form.

WATER

The problems of water supply are similar for both sand and water cultures. The points to be examined include sources and composition of natural and purified water supplies, method of purification and storage, toxicity, and the suitability and uses to which various types of supply are adapted.

Perhaps this section may be aptly prefaced by the opening comments of Woodward (1699) : " Some waters are indeed so very clear and transparent that one would not easily suspect any terrestial matter were latent in them : but they may be highly saturated with such matter tho' the eye may not be presently able to describe or discern it . . . upon the whole it is palpable and beyond reasonable contest that water contains in it a very considerable quantity of terrestial matter . . ." The warning is as relevant now to the consideration of distilled water as it was in Woodward's time to that of other water supplies.

Sources of Water

Natural sources of water comprise sea water, ground water from rivers and wells, and rain water collected on a prepared or artificial surface and stored in tanks. Artificial or modified sources include tap water which is often chlorinated or softened and is conducted through metal conduits ; demineralised water, and distilled water.

Sea water would be unsuitable for most purposes, and its high concentration of salts (exceeding 1,000 p.p.m.) renders it toxic to most plants. No records have been found of its use in culture experiments. River and well water may be hard or soft according to the district and geology. Water from upland streams is likely to be non-toxic, but river water may be polluted by organic sewage and heavy metal contaminants. Rain water may contain sufficient of most nutrients for the slow growth of trees as found by Duhamel du Monceau (1758) for water from the Seine. Knop (1860) raised seedlings on spring water prior to adding the nutrient salts. Uncontaminated calcareous stream water has been used successfully for work on manganese deficiency in soil cultures by Portsmouth (1947 unpublished). Sources of clean river water, when treated by demineralisation procedures described below (1. c.), should present useful possibilities in large-scale projects, for outposts and regions where tap water or adequate rain water is not available. " De-salted " water prepared by the Siemens electro-osmosis method was used in work on absorption by Humphries (1950).

Rain Water

Composition of rain water. The mineral nutrient content of rain water (including nitrogen) varies considerably according to location, season and means of collection. Early work up to 1905 on the composition of rain water (and especially the nitrogenous and chloride content) has been extensively reviewed by Miller (1905). Crowther and Steuart (1912) found that only 13 of 157 samples of precipitations collected around Leeds were acid, the remainder being neutral or alkaline. MacIntyre and Young (1923) similarly found that all samples of rain collected in Tennessee, whether in a town (Knoxville) or 1½ miles out, were alkaline to methyl orange. Rain water collected at Long Ashton has a pH of 5-6·5.

Solid precipitation carried down by rain is a significant fraction of the total impurity, especially near towns. Crowther and Ruston (1911) estimated that solid precipitation was 1,100 lb./acre annually in the centre of Leeds and 50 lb./acre 4 miles out. The latter figure corresponds to 4 lb. per annum on the roof of a glasshouse covering 3,500-4,000 sq. feet. This surface may collect 40,000 gallons of water in about 9 months and thus may include up to 8 p.p.m. partly soluble solids in the water due to solid precipitation. This estimate of contamination gives some indication of the lower limits of purity to be expected in rain water collected near but outside a large town. A detailed study of atmospheric pollution in relation to proximity of a town, prevailing wind and time of year has been published by Dobson (1945). The pollution of rain water is greatly reduced at distances from 1½ to 4 miles away from towns according to Crowther and Ruston (1911), Crowther and Steuart (1912),MacIntyre

and Young (1923), and Dobson (1945). The content of chloride present is considerably affected by prevailing wind and proximity to the sea. At Rothamsted, about 50 miles from the East coast of England, the mean chloride content is 2·1-3·4 p.p.m., Miller (1905), and 218 p.p.m. at Land's End, according to Franklin (cited by Miller (1905)). The nitrogen content of rain water comprises ammonia and nitrate fractions and occasionally smaller amounts of organic nitrogen, Miller (1905), Crowther and Ruston (1911). The total nitrogen levels range somewhat widely according to data given by Miller (1905), Crowther and Ruston (1911) and Collison and Mensching (1932), from about 0·6-2·8 p.p.m. from widely separated areas in Great Britain and the United States having annual rainfall between 19 and 48·5 inches. The bulk of nitrogen is present as ammonia. The sulphur content of rain water shows the greatest variation according to proximity of towns and railways. Close to these sources of contamination the content may average 5-23 p.p.m. In the country the average is around 0·6-1·9 p.p.m. according to Miller (1905), Erdman (1922), MacIntyre and Young (1923) and Collison and Mensching (1932). Demolon and Bastisse (1941) found a range of 0·57-6·18 p.p.m. sulphur in rain water collected at various times in France, and the maximum occurred after dry periods.

The composition of rain water varies with time of sampling and amount of rainfall at any period. Winter rain generally carries down more chlorine than summer rain, as was found by Crowther and Ruston (1911), and Collison and Mensching (1932), but the correlation with seasons is less consistent for sulphur and nitrogen. The first increment of rain falling during any period is usually more contaminated by solid and soluble impurities according to Collison and Mensching (1932), since the dust in the atmosphere is precipitated with the initial fall. Similarly, light rain is more contaminated than heavy rain. The total nitrogen content of rain at Rothamsted was found by Miller (1905) to be inversely related to the amount of rainfall in each month. Crowther and Ruston (1911) found that light rainfall up to 1″ per month contained 6·9 p.p.m. Cl., whereas heavier rain, exceeding 4″ per month contained only 2·2 p.p.m. Cl.

There are few data on the mineral content other than nitrogen, sulphur and chlorine present in rain water. These lesser known fractions, however, are probably of equal or even greater importance when the suitability of rain water for nutritional experiments is considered. The data in Table 9 summarise the information for some of these elements obtained from a number of sources, and Tables 10 and 11 show results obtained by the writer over a period of about 7 years for water collected from a glass roof at Long Ashton situated about 3 miles from Bristol and representative of a centre in a rural area close to a fair sized town. The data in Tables 10 and 11 show that approximately 0·07-2·72 p.p.m. calcium, 0·05-0·56 p.p.m. magnesium and 0·5-3·0 p.p.m. potassium may be expected in rain water collected under these conditions. The micronutrient content of rain water is fortunately relatively low, much of the contamination being caused by organic debris on the glass roof and gutter of the greenhouse and by the accumulation of small amounts of coal ashes from a neighbouring railway. Coal ash is notably rich in several micronutrients and other rarer elements, as determined by Goldschmidt (1936) (1943). Edelman (1939) ascribed the boron content of rain water mainly to the presence of cyclic salt in maritime areas. The importance of sulphur in rainfall has been stressed in the United States of America where sulphur deficiency occurs only in areas remote from large towns. The magnitude of this source of sulphur will be of significance when rain water is used in culture experiments.

Seasonal variations in the content of several mineral elements are noteworthy. The majority tend to accumulate and this trend may be explained partly by storage contamination and partly because the first samples usually represent water collected after heavy rainfall, whilst later samples represent the accumulation of smaller additions during the drier part of the year. Manganese frequently decreases, however, and this may be due to oxidation to hydrated manganese dioxide which is precipitated and partly eliminated by the raised outlets from the tanks (see Part Two). The seasonal decreases shown in 1946 were probably due to unusually heavy rainfall during much of the summer season that year.

TABLE 9

Miscellaneous Data on Mineral Content of Rain Water Excluding Chlorine, Nitrogen and Sulphur

Reference	Location	PO_4	Ca	Mg	K	Na	Fe	Al	Mn p.p.m.
Lawes, Gilbert and Warington (1881)	*Rothamsted*	—	(Ca+Mg) Carbonates 4·0 (Ca=2·4 p.p.m.)						
MacIntyre & Young (1923) ...	(*Tennessee*)								
(Recalculated data)	*Knoxville City Centre*	Trace	4·3	0·5	4·8	3·5	Trace	Trace	Trace
„ „	January-March								
	April-June	—	1·4	0·4	1·2	0·6			
	1½ miles from *Knoxville*								
„ „	January-March ...	Trace	0·8	0·6	1·7	1·2	Trace	Trace	Trace
	April-June	—	0·8	0·2	1·0	0·4			
Wallace (1924)	*Long Ashton*								
	Glass Roof	0·03	1·4	0·36	0·25	—	(Sulphur 1·2)		
	Tiled Roof of Lab.	—	4·5	0·9	—	—	(Sulphur 4·0)		
Bertrand (1945 a b,)	*Paris*	—	(Ca about 1·8) Ca/Mg ratio 10 : 1	0·18	0·2-0·4	—			
Bakker (1946)	*Fécamp* 1850 ...	—	5·8	—	—	11·9			
Private communication of data from several sources	*Utrecht* 1933 ...	—	3·3	0·7	—	4·9			
	Caen (kg/hectare/annum)	—	2·8	1·5	8·0	18·0			
	Calculated p.p.m. for 30″ rainfall ...	—	1·0	0·55	2·9	6·5			

Table 10

Analyses of Rain Water obtained at Long Ashton (Hewitt, unpublished data)

Sample and Description and Date	p.p.m. Ca	Mg	K	Na	Fe	Mn	Cu	Zn	B	Mo
Rain water from gutter during rain 14/5/43 ...	—	—	—	—	·055	·011	·015	·039	—	—
West storage battery of *old* galvanised tanks unpainted 2/4/43	—	—	—	—	—	—	—	12·0	—	—
Ditto East battery	—	—	—	—	—	—	—	10·0	—	—
Sample from small galvanised tank in greenhouse filled from above 19/4/43	—	—	—	—	·03	·019	·03	10-13	—	—
East and West batteries. Composition sample *after painting with bitumen* 14/5/43	—	—	—	—	·028	·010	·014	·4-·5	—	—
Small tank in greenhouse (unpainted) 6/10/43 ...	—	—	—	—	·067	·005	—	2·94	—	—
West battery 19/6/43	·20	·50	—	—	—	—	—	1·61	·0055	—
1944 *onwards. All galvanised tanks cleaned and repainted with 2 coats annually*										
Sample from East battery 24/ 4/44	·11	·05	—	—	·067	·023	·01	·21	·006	—
Ditto 10/10/44	2·1	·56	·5	—	·08	·01	·01	·35	·030	—
Sample from East battery May, 1945	·07	·14	1·5	—	·096	—	·012	—	·004	—
Ditto September, 1945 ...	2·2	·14	3·0	—	·35	—	·012	·20	·040	—
Sample from West battery October, 1945	—	·11	—	—	·11	—	—	·15	·035	—
Sample from East battery 9/ 1/46	—	—	—	—	·025	—	—	—	—	—
Ditto 4/ 5/46	2·72	·26	—	1·01	·10	·016	—	·44	·006	·0013
Ditto 24/ 6/46	—	—	—	—	·080	·02	—	—	—	—
Ditto 17/ 8/46	1·90	·19	—	—	·093	·025	·018	·465	—	—
Ditto 1/10/46	1·04	·104	—	—	·07	·022	·017	·54	·007	·00055
Ditto 20/12/46	—	—	—	—	—	·01	—	—	—	—
Sample from new battery. New galvanised tanks painted with bitumen paint and filled from East battery 4/4/46. No water entered these tanks after 4/4/46	—	—	—	—	·10	·0085	·044	·95	—	—
New battery 17/8/46	—	—	—	—	·065*	—	·050	2·44	—	—
Sample from West battery 4/5/46	1·62	·25	2·09	1·33	—	—	—	—	·008	—
Ditto 17/8/46	1·68	·13	1·65	·525	—	—	—	—	·009	—

* A sample collected after stirring up sediment in residual 2″ depth of water at bottom of tank contained 0·33 p.p.m. Fe.

TABLE 11

Summary of Concentrations in Parts per Million of Nutrient Elements Found in Rain Water at Long Ashton

	Na	K	Ca	Mg	Fe	Mn	Cu	Zn*	B	Mo†
Average ...	0·95	1·75	1·42	0·18	0·08	0·016	0·027	0·52	0·013	0·00075
Minimum ...	0·53	0·50	0·07	0·05	0·025	0·005	0·010	0·15	0·004	0·00018
Maximum ...	1·33	3·00	2·72	0·56	0·35	0·025	0·062	2·94	0·040	0·0013

* The figures for zinc all apply to painted tanks.

† The minimum figures for molybdenum apply to results obtained by microbiological assay with *A. niger*.

The principal conclusions to be drawn from this survey of mineral contamination in rain water are : that the bulk of water should be collected, if possible, during periods of heavy rain ; rain water, even before storage, has an appreciable mineral content, but this is low enough for many purposes including the study of many mineral deficiency effects ; that a device such as that described by Thresh (1896) might be advantageous for discharging to waste the first fraction of each fall of rain before collection.

Storage of water. The storage of water presents certain problems which are especially important when rain water is concerned. Galvanised tanks and equipment may give rise to concentrations of zinc in the water injurious to several plants, as shown in Table 12.

TABLE 12

Concentrations of Zinc Causing Injury to Culture Plants

Reference	Conc. of Zinc	Service	Crop Injured
Chapman *et al.* (1939) ...	30 p.p.m. ...	from galvanised pipe	Citrus
Early (1943)	1·6 p.p.m. ...	from galvanised pump and piping ...	Soya bean
Harris (1946)	6 p.p.m. ...	from galvanised tank ...	Green algae
Long Ashton	12-20 p.p.m. ...	unpainted galvanised tank	Sugar beet and turnip

The accumulation of zinc can be greatly reduced by painting galvanised tanks with a pure bitumen paint. The zinc content may be 2-3 p.p.m. after 6 months' storage in painted *new* galvanised tanks and as little as 0·2 p.p.m. after 9 months in painted *old* tanks. It is important that galvanised tanks should be well weathered by exposure before applying any paint. Failure to observe this precaution leads to continued solution of zinc through the paint which is frequently destroyed by the presence of flux compounds, (usually ammonium chloride), remaining after galvanising especially in the region of seams, angles and rivets. Harris (1946) found that less zinc dissolved in water from a weathered tank than from a new galvanised surface. The treatment given at Long Ashton before painting is outlined in Part Two. Evans (1946) recommends the following treatment of galvanised surfaces before applying bitumen paint :—

(a) The surface must be well weathered.

(b) The surface is roughened by brushing with the following mixture :—

Hydrochloric acid	5 volumes	
Carbon tetrachloride	5	„
Toluene	30	„
Methylated spirit	60	„ and then wiped dry.

For the coating of the inner surfaces of pipes the Talbot process (Reddington *et al.* J. Amer. Waterworks Ass. 1940, 22 ; 1385), Evans (1946 1. c.) is recommended. Two thin coats are more effective than a single thick coat as there is a greater likelihood of covering over small perforations. Further details regarding the painting of galvanised iron are given by Duncan (1935), who also emphasised the importance of weathering for a year or two before painting. Causes of failure were ascribed to grease, zinc chloride or the nature of the galvanised surface. A suitable treatment to be applied before painting would comprise washing with acetic acid, a grease solvent and detergent.

Other factors hastening the breakdown of bitumen-painted surfaces include ultra-violet light, sudden cooling and particles lying on the bottom of the tank. Soft water is far more corrosive to galvanised surfaces than hard water according to Evans (1946). Bitumen paints and solutions were found frequently to contain iron, " lime " and also copper by Goldsmith (1943) and Evans (1946). It is important to select a pure bitumen solution of not less than 99% organic matter. Analyses of bitumen used at Long Ashton are given in Part Two. on containers. Further details regarding bitumen preparations are given by Preston (1947). To improve adhesion these materials may contain metallic soaps including salts of aluminium, iron and the alkaline earth metals. Fillers containing aluminium, chromium, zinc, lead, iron or copper are often incorporated. Preston (1947) stated that bitumen is about 20 times as impermeable to water as paraffin wax, but protection and impermeability are reduced by immersion and oxidation which is particularly catalysed by light. The browning that occurs in a bitumen surface, however, is due to partial oxidation and may arrest further breakdown. Damp surfaces cause poor adhesion.

Other materials for storage tanks include glass, concrete and black iron, which should be shot-blasted to remove mill scale before applying bitumen paint according to Evans (1946), and iron tanks treated by application of a baked synthetic resin surface.* Small tanks of this type have been examined, Hewitt (unpublished data) and accumulation of some micronutrients, although greatly reduced, was still detected. A similar test was made on a welded black-iron tank lined by spraying with polythene. The rates of accumulation in 4 gallons of distilled water stored in such tanks for a period of 6 months were as follows :—

(Accumulation as mg. per square foot of immersed surface)

	Fe	Mn	Zn	B	Mo
Synthetic resin-lined tank* ...	·06	·012	·144	·006	·0006
Polythene spray-lined tank ...	·73	·08	2·32	·008	·011

The use of bitumen-painted black-iron containers for plant cultures has been discussed in the Section on containers.

Untreated concrete tanks were used by Vogel (1929) (1931) and bitumen-lined concrete was used by Brown (1945) to store rain water.

Glass storage vessels are satisfactory for many purposes, but slight accumulation of iron may occur even from glass carboys, formerly used to store mineral acids. Pyrex glass storage vessels, although expensive, probably cause least contamination except from boron. The data presented in Table 14 on demineralised rain water discussed below suggest that their use may be justified in some instances. Culture experiments and analyses (reported in

* Supplied by the Prodorite Company, Eagle Works, Wednesbury, Staffs, to whom grateful acknowledgment is made.

Part One on containers) indicate that polythene sheet may be useful as a lining material for large metal storage tanks. Thomas *et al.* (1943 a) used plate glass-lined large concrete or iron tanks for storage purposes in other work referred to under large scale installations.

Consumption. The size of storage tanks needed is related to consumption and naturally depends on the number and type of cultures and the expected rainfall. Thomas *et al.* (1943 b) found a mean consumption of 8 gallons per day for each sand bed $6' \times 2'\ 6''$. Davidson (1946) estimated that storage tanks should hold 2 gallons per square foot of sand bed. Hewitt (unpublished data) found that for separate pot sand-cultures under glass the mean consumption per pot for about 5 months was about 10-20 gallons according to treatment. 7,000 gallons storage capacity for rain water should be allowed for 1,200 cultures with a summer rainfall of about 12-15 inches. Davis (1930) found that 3-year-old Bramley's seedling apple trees grown in 20-inch pots required 98 L. solution and 30 L. extra water for leaching during a season's growth. The quantities required for 12-inch pots were 85 L. and 20 L. respectively. Eaton (1936) found that 4-year-old apple trees used 100 L/day in transpiration when grown in tanks $10 \times 3 \times 6$ ft. and 60-70 L/day were lost from shallow beds 12 feet $\times$ 18 inches used to grow vegetables.

Use of rain water. Rain water has been extensively used by many workers especiallyWallace (1922), (1924), (1928), (1930) and Davis (1930) in experiments on macronutrient deficiencies and excesses in apple trees in sand culture, by Vogel (1929), (1931) for large-scale cultures and by Brown (1945) on peach trees. Hewitt (1944), (1945), (1946 a, b), (1948 a), (1949 a, b), used rain water in extensive large-scale experiments with vegetables. The results obtained in these experiments have shown that rain water, suitably collected from a clean surface such as a glass roof and stored in suitably treated containers (see Part Two and data below on storage and analysis, etc.) is satisfactory for a great many purposes. These include the study of effects of macronutrient deficiencies and excesses on vegetable and fruit crops, boron deficiency (and toxicity) effects on several crops with moderate or high requirements such as Brassicas, beet, tomato and leguminous crops. Clean but unpurified rain water suitably collected and stored is not injurious, but analyses given below show that rain water might not be satisfactory for work with high rates of continuous flow of solutions containing only low levels of potassium, calcium or phosphorus of the order of 2 p.p.m. or less (see Section on minimum levels of nutrient supply), for precise work on iron and manganese deficiency, or for work on nitrogen fixation or excretion by bacteria and legumes in sand cultures. Lack of response to sulphur deficiency in early experiments by the writer at Long Ashton may have been due to the presence of sulphur in the rain water used. No information is at present available with respect to the suitability of untreated rain water for work on copper, zinc or molybdenum deficiencies, but analyses (Tables 9, 10, 11) suggest that it could not be used for these purposes. Evans (private communication) found that rain water collected from a plastic cloth was the purest source of boron-free water available.

Tap Water

Tap water varies more widely in composition than rain water and water from the same source may show considerable variation in, for example, calcium content from day to day. Analyses of tap water supplies are given below in Table 13, and indicate the probable mineral content that may be expected. In spite of the range in mineral content, tap water has been used in many experiments. It is often suitable for toxicity studies and work on salt effects, as, for example, aluminium toxicity (McLean and Gilbert 1926) ; boron or salt tolerance (Eaton 1942 a, 1944) ; iodide toxicity (Lewis and Powers 1941) ; for the production of deficiencies of nitrogen, phosphorus and possibly potassium (Houghland 1947) ; for large-scale cultures with fruit trees (Hayward *et al.* 1946 and Read 1947 (private communication)) ; studies on manganese and aluminium toxicities and on types of sand for use in sand culture (Hewitt 1947 b ; 1948 a and unpublished) ; and the interrelationships of iron deficiency and nitrogen supply in rice (Asana 1945). McLean and Gilbert, Lewis and Powers, Asana, and Houghland grew their plants in water culture, the others cited used sand cultures.

TABLE 13

Analytical Data for Tap Water Samples Used in Culture Experiments

Reference	NO_3	SO_4	Cl	Ca	Mg	K	Na	Fe	Mn	Cu	Zn	B	p.p.m. Mo
Arndt and Hoffman (1937)	—	—	—	—	—	—	—	—	—	0·04	—	—	—
Eaton (1944)	—	—	—	—	—	—	—	—	—	—	—	·03—·04	—
Hewitt	—	—	—	40-110*	11-14	K + Na 11·0		·04-·08	·008	—	—	·053—·07	·0003†
Liebig *et al.* (1943 a) ...	8·0	28·0	21·0	48·0	6·0	3·0	24·0	·05	·001	·008	Not detected	0-1	·01 (Vanadium ·1)
Hayward and Long (1943)	—	12·0	16·0	28·0	—	2·0	34·0	—	—	—	—	—	—
Hayward *et al.* (1946) ...	1·86	25·9	14·6	35·8	5·42	3·12	32·2	—	—	—	—	—	—

* Calcium content at Long Ashton varies between limits indicated.

† Determined by *A. niger* method.

The presence of free chlorine in tap water might be injurious but chloride tolerance of plants appears greatly to exceed the combined chloride content likely to occur in tap water according to Lewis and Powers (1941) and Eaton (1942 a). Zimmerman and Berg (1933) and Withrow and Withrow (1948) considered that the usual concentrations of *free* chlorine were unlikely to exceed the toxicity threshold of approximately 5 p.p.m., except where aquatic plants are grown, as these are much more sensitive. Martin (1935) found that whilst sugar cane was injured in solutions prepared with tap water at pH8 no injury was produced with tap water acidified to pH6.

Tap water is frequently slightly alkaline and the pH may even exceed 8·0. Eaton (1941 c), Hayward and Long (1941 b) and Gauch and Wadleigh (1945) used nitric acid to reduce to a suitable value the pH of nutrient solutions made up with tap water. The nitric acid added is thus used to supply about 2-3 mg. eq./L. of nitrate as well as serving for pH control. The writer has found that the addition of sulphuric acid for reduction in pH may cause precipitation of concentrated stock nutrient mixtures on standing due to slow decomposition of bicarbonate, and the initial pH value produced in addition to the acid is not stable.

Tap water was used without further treatment for the production of manganese deficiency by Miller (1928), in tomato, barley, wheat, maize, cabbage and tobacco grown in sand culture. Tap water available at Long Ashton contains only ·008 p.p.m. Mn. Liebig *et al.* (1943 a) found only ·001 p.p.m. in their supply and such water can be used to produce manganese deficiency in many plants.

Tap water does not generally seem to be suitable for production of boron deficiency. Thus Mazé (1915) found that growth of maize ceased suddenly in a few weeks when a nutrient devoid of additional boron was made up with distilled water, but no such effect occurred when tap water was used. McMurtrey (1929), Löhnis (1937) and van Schreven (1939) similarly found that tap water delayed or prevented the appearance of boron deficiency in several plants which was acutely noticeable with distilled water. Schroeder *et al.* (1946) obtained results with table beet from which similar conclusions may be drawn. Eaton (1944) found that tap water was sufficiently low in boron for deficiency effects to occur in beet, turnip and cotton, but not in many other plants. Analyses made at Long Ashton indicate that a boron content of 0·05 p.p.m. may be about the threshold values for many plants.

The molybdenum level of tap water at Long Ashton is about ·0003 p.p.m. which may suffice for some plants. Jensen and Betty (1943) found only ·000055 p.p.m. molybdenum as well as 0·1 p.p.m. of manganese and vanadium and traces of several other heavy metals in tap water.

Distilled Water

Distilled water is widely used for nutrition experiments and reference to the numerous examples of particular work where distilled water has been used is unnecessary except to illustrate special points of interest. This section will deal with the purity and occasional contamination found in distilled water, the design of stills and the suitability of water from different types of stills, and the special precautions recommended for obtaining water of great purity with respect to mineral elements.

Distilled water is probably the purest source of water available, but repeated treatment by passage through synthetic resin ion-exchange equipment of suitable design may yield an effluent comparable even to glass-distilled water, as will be shown in the next section. Stills can be made from three basic materials, namely metal, glass and quartz, and these materials may be combined in one still so that a metal boiler is attached to a non-metal condensing surface.

Metal stills. There are two types of all-metal stills of interest for nutritional experiments. The simplest is the tinned copper still with a pure electrolytic tin (or frequently a heavily tinned copper) condenser. Several patterns of continuous operating stills of this type are available. They can be run from gas, steam, electricity or oil and have outputs of from 3 to 50 or more litres an hour. It is necessary to include a water softener in the circuit when even

only moderately hard water supply is used, otherwise the boiling pan and condenser become coated with scale and the rate is greatly reduced. Experience at Long Ashton suggests, however, that a thin coating of scale may materially assist in reducing metal contamination. Newly installed plumbing with unions that have been made with linseed oil and red lead may also be a cause of contamination as the oil may cause surface " creep " in the stills for a long period and the fault is hard to trace. The probable micronutrient content of water from carefully operated tinned copper stills in good condition supplied with softened tap water is shown by the following figures (as parts per million) obtained at Long Ashton :—

Fe ·004-·006 ; Mn ·004 ; Cu ·014 ; Zn ·0075 ; B ·003 ; Mo ·0003-·0006.

Additional data given by other workers show that " ordinary laboratory distilled water " may contain considerably more copper and zinc than shown above. The following figures as p.p.m. have been recorded : Broyer and Furnstall (1945) : Cu ·01-·05 ; Piper and Oertel (1941), Piper (1944) : Cu ·02-·07 ; Zn ·013-·04 ; Arndt and Hoffman (1937) : Cu ·07 ; Trumble and Ferres (1946) : Cu ·1 ; Guest and Chapman (1944) : Cu ·11 ; Liebig *et al.* (1943 a) : Cu ·2 ; Zn ·02 ; Pb ·055 ; Walkley (1942) : Zn ·03. Other metals present in appreciable amounts in metal distilled water in addition to Fe, Mn, Cu, Zn, B and Mo, may include As, Bi, Ni, Pb, Sn, even when " pure " block tin condensers are used, as, for example, by McHargue and Offut (1940). Distillation in such stills is also likely to increase the copper contamination in the water as found by Arndt and Hoffman (1937), and the writer. Hewitt and Bolle-Jones (unpublished) showed that tinned copper stills with pyrex glass condensers were totally unsatisfactory for work on copper and zinc deficiencies.

Distilled water from tinned copper stills is satisfactory for many experiments with boron deficiency and allowed very severe deficiency effects to develop rapidly in a wide range of plants grown by Hewitt (1945), (1946 a), (1947 a) ; Eltinge (1936) produced boron deficiency symptoms in maize in water culture in 5-7 days, with water from tinned copper stills, and Smith (1943) also found this water satisfactory for boron deficiency.

Water from such stills has been used for work on manganese deficiency by Arnon (1938), Stout and Arnon (1939), Piper (1944) and Hewitt (1945), (1946 a), (1947 a), and acute deficiency effects were readily induced in many plants by using such water. The use of distilled water condensed in quartz for work of this nature with higher plants by McHargue and Calfee (1932 b) and by Hageman *et al.* (1942), seems an unnecessary refinement, especially when the water was stored in stoneware jars.

The suitability of water from tinned copper stills for work with molybdenum deficiency is doubtful. Arnon and Stout (1939 b) stated that ordinary distilled water usually contained traces of molybdenum sufficient for plant growth and that water condensed in pyrex glass is necessary for consistent results. Steinberg (1945 a) on the other hand, considered that redistillation of water was not necessary, as most molybdenum contamination was due to the reagents, but no comparative data were given. Microbiological assays with *A. niger* by Hewitt and Hallas (1951) indicate that the molybdenum level may be about the threshold concentration (see Table 18 and below). Evans *et al.* (1950) stated that " ordinary distilled water " contained 0·0001 to 0·00008 p.p.m. molybdenum.

Wagner (1940) claimed to produce deficiency effects in beet in water culture due to lack of silicon. The water was obtained by redistillation from tinned copper stills, but no extra zinc or molybdenum was given in these experiments.

It is not only clear that water from tinned copper stills is quite unsuitable for the production of copper and zinc deficiencies as confirmed by Arnon (1938), Stout and Arnon (1939) and Piper (1944), but toxicity effects may be induced in water cultures by using water from such stills. This was found by Liebig *et al.* (1942) and Guest and Chapman (1944) with citrus and by Broyer and Furnstall (1945) with barley. The latter found that toxic effects were induced when unpurified salts and water from tinned copper stills were used, and together these contributed ·05-·08 p.p.m. Cu. When water was stored in tinned copper containers there was an accumulation of ·17-·2 p.p.m. Cu. The concentrations of copper and zinc noted above are in the range of toxicity found for *water* cultures by Brenchley (1910),

(1914) who showed that about ·05 p.p.m. Zn and ·1 p.p.m. Cu are about the threshold concentration for toxic effects on pea and barley respectively.

The possibility of toxic constituents occurring in water from tinned copper stills was the reason for the introduction by Brenchley (1914), (1916) at Rothamsted of stills with solid silver condensing surfaces. These stills are similar to the standard pattern,* but the steam guide, condensing cone, gutter and outlet are all of solid silver. Only pure silver solder free of lead should be used in the fabrication of the parts. The boiling pan is of tinned copper and may be heated by gas or electricity. These stills have supplied all distilled water used in the water culture work at Rothamsted (Brenchley private communication). The output is about 3·5-4 litres an hour. Water from these stills is entirely suitable for work on boron deficiency and probably manganese deficiency. Responses to molybdenum deficiency have also been obtained with lettuce by Warington (1946). Similar stills installed at Long Ashton produce a distillate which contains 0·00006 p.p.m. Mo and also gives a weak reaction for heavy metals with diphenylthiocarbazone.

Metal stills are normally able to give a much greater output than glass stills owing to the larger capacity and area of the boiling pan and condenser. Some of these advantages may be combined with a condensate produced in pyrex glass or quartz condensers. A standard commercial model electrically operated with an output of 3 litres per hour is available.† Special stills for large output and high quality of water have been designed by Arnon (private communication) and by McHargue and Offut (1940). The arrangement used in Arnon's still is shown in Figure 1. Steam at a pressure of 80-100 lbs. per sq. inch in a steam coil is used to boil water in a tinned copper boiling pan. A constant level is maintained in Arnon's still by feeding once-distilled water from tinned copper reservoirs arranged so that a ball valve admits water to the reservoir when the level in it falls due to corresponding fall of level in the still. The vapour is led through a tall column of pyrex glass fitted with a splash bulb some distance from the boiling pan before passing over to the condenser. The condensate is stored in pyrex glass carboys and each batch is tested separately by the dithizone test, specified by Stout and Arnon (1939). This test will detect as little as 0·2 microgram of total heavy metals in 200 ml. of water. A distillate with a total heavy metal content of 0·002 p.p.m. or less is consistently obtained. Water from this still is satisfactory for work on copper, zinc and molybdenum deficiencies.

The still designed by McHargue and Offut (1940) was arranged so that the hot vapour left the boiling pan by a side arm connected to a porcelain dome and baffle. These were connected with five quartz tube condensers. The distillate was stored in acid-resisting glazed stoneware and had a total metal content of ·002 p.p.m., but did not give a positive result with the dithizone test used by Stout and Arnon (1939). The output was 13-15 litres an hour.

Non-metal stills. Several non-metal stills have been described. These stills give the most consistently pure distillate, but the rate is usually low and may be between ·5-3 L. per hour. For large-scale work several are therefore needed. It is also desirable that the stills should be reliably designed to allow a continuous supply of water to the boiling vessel. Owing to the small capacity, breakage due to running dry is a serious hazard.

The usual materials are glass and quartz, and the latter was used as early as 1917 by Maquenne and Demoussy. Pyrex glass was considered to be the most suitable type of glass available by Sommer and Lipman (1926), Sommer (1931), Steinberg (1936), (1938 b), Stout and Arnon (1939), Piper and Oertel (1941), and Piper (1944) (and private communications), except, of course, where boron or silicon deficiencies are concerned. Schropp and Arenz (1940) used a still made of soda glass for boron deficiency. Other types of glass such as " resistance glass " may give rise to considerable amounts of zinc according to Walkley (1942), but hysil glass appears to be as suitable as pyrex. Earlier work in Germany was frequently carried out with water obtained from Jena " Gerete 20 " glass as for example by Fitting (1928), Roberg (1928), (1931) and Gollmick (1935). Jena glass was definitely

* Messrs. P. Brown & Co., Alembic Works, London.

† Messrs. Baird and Tatlock, Ltd., Chadwell Heath, Essex.

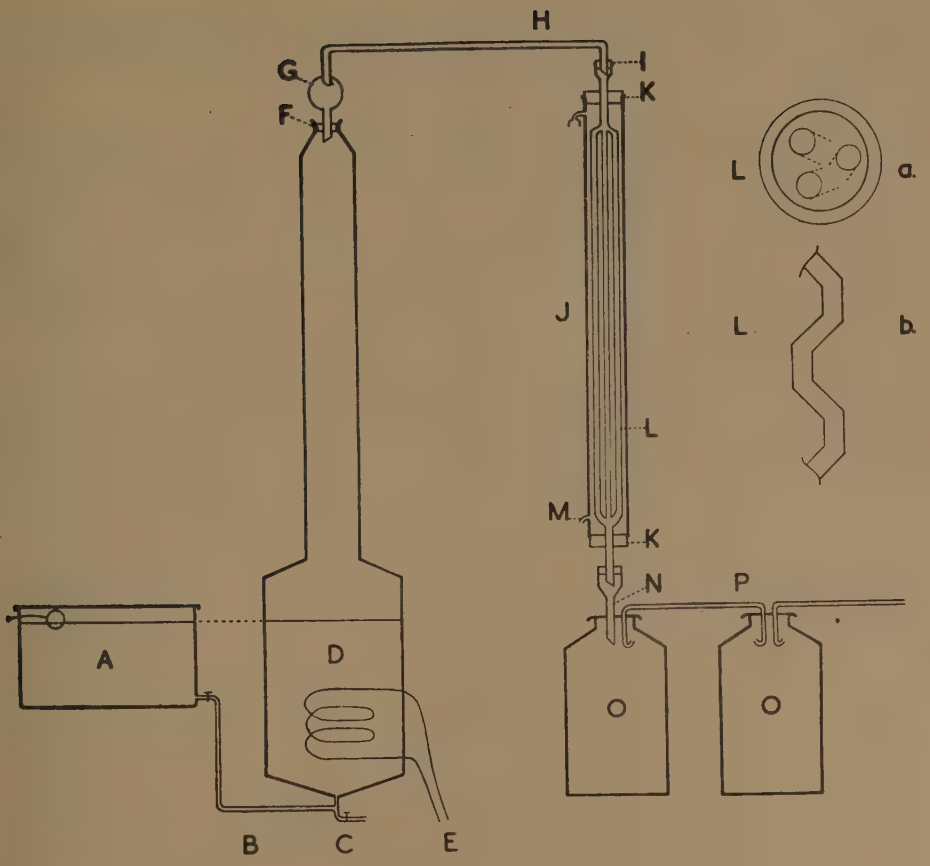


FIGURE 1

Large-scale still used at University of California for production of copper and zinc-free water. Recommended output 6 L.p.hr. (After Arnon and Furnstall, unpublished data).

A = Tinned copper reservoir, 46 × 26 cm., and ball valve.
B = Water inlet.
C = Drainage cock.
D = 10 gal. tinned copper still, 36 cm. wide at base, 226 cm. high.
E = Steam coil, 60-90 lb. p.s.i.
F = Cork.
G = Splash trap.
H = 20 mm. pyrex glass bend (length 76 cm.).
I = Cork or ground glass.
J = Pyrex glass condenser, 165 cm. high.
K = Rubber bung.
L = 3 zig-zag tubes (see also enlarged sections a and b).
M = 10 mm. water inlet.
N = Delivery tube.
O = 5 gal. pyrex glass collecting jars.
P = 10 mm. connecting siphon.
aL = Enlarged horizontal section of pyrex glass condenser showing 3 cooling tubes.
bL = Enlarged vertical section showing zig-zag arrangement of cooling tube.

inferior to quartz according to Gollmick (1935), but the results of Roberg (1932) were inconclusive. There appears to be little difference between pyrex glass and quartz with respect to copper, zinc and molybdenum according to Steinberg (1936), but the difference may not be entirely negative for zinc according to Walkley (1942).

Water redistilled in all glass or quartz stills may not, however, be entirely free of heavy metals. These may be entrained in spray if adequate splash traps are not provided, and even when these are present contamination may still occur. This contamination is much greater at the commencement of distillation than later, even when clean well used stills are considered, according to Piper and Oertel (1941) and may give a marked positive reaction with the dithizone test, (Hewitt unpublished data). The cause of this contamination is probably due to " creep " along the wet surfaces which continues until they are at the same temperature as the steam in contact with them. To meet this difficulty of initial contamination it is customary to reject the initial distillate of 50-200 ml. as was done by Fitting (1928), Roberg (1928), (1931), and Piper and Oertel (1941).

The problems of entrainment and possibly of " creep " have been approached by the inclusion of splash traps of various types such as those used by Fitting (1928), Roberg (1931). Piper and Oertel (1941), Hewitt—shown in Part Two—, Cliquet *et al.* (1935) and Waring and Werkman (1942) relied on a long column. Roberg (1931) found no improvement when a second splash trap was inserted but on theoretical grounds entrainment should be greatly reduced by a double trap. Roberg (1931) referred to Hahn (1922) and Rapp (1928) who used a column of glass rings to " scrub " the vapour and this device was also adopted by Roberg (1931). No splash trap was provided by Liebig (1940). There is considerable scope for further study of the design of efficient splash traps and the prevention of entrained spray. Hutner *et al.* (1950) have suggested using a non-metallic essential growth factor such as p. amino benzoic acid to test the " carry over " from entrained spray in stills. A radioactive isotope would still more effectively test the behaviour of metal compounds in stills.

The provision of dust traps is an important point provided for by Fitting (1928), Piper and Oertel (1941) and Hewitt (Part Two).

Many experiments on manganese, copper, zinc and molybdenum deficiencies have been made with water obtained by a *single* redistillation, in an all-pyrex glass still, of water produced in metal stills, as, for example, in the work of Sommer and Lipman (1926), Sommer (1931), Chapman *et al.* (1937), Piper (1940), (1941), (1942), Pearse (1944) and Hewitt and Jones (1947), (1949), (1950). Javillier (1907) used water distilled from glass under reduced pressure for early work on the zinc requirement of *Aspergillus niger*. The use of reduced pressure, however, was not favoured by Cliquet *et al.* (1933) owing to great entrainment of spray and increased resistance to condensation.

For the most refined and precise experiments, particularly with copper deficiency, a second redistillation from pyrex glass is considered necessary by Piper (private communication). Water twice redistilled from pyrex glass has been used for work on zinc and copper deficiency by Brandenburg (1933), van Schreven (1936 a), Arndt and Hoffman (1937), Millikan (1942) (1944), Smith and Bayliss (1942), Wade (1944), Hewitt and Bolle-Jones (unpublished) and for molybdenum deficiency with *Aspergillus niger* by Hewitt and Hallas (1951).

Distillation and redistillation from all-quartz stills have been used in some experiments. Thus Steinberg (1938 b) used water distilled in turn from metal stills, pyrex glass and finally quartz stills in the experiments where he found a response to gallium by *A. niger*. This was, however, not confirmed in later work (Steinberg 1945 a). Steinberg (1945 b) used water twice distilled from quartz for experiments in which *Lemna minor* was used as a test organism for several micronutrients ; marked responses were obtained with deficiencies of iron, manganese, boron and molybdenum and less pronounced effects with copper and zinc. Roberg (1931), (1932), used distilled water obtained by double distillation from Jena glass followed by further distillation from quartz to study zinc deficiency in *A. niger* and blue-green algae. Waring and Werkman (1942) found it necessary to use water distilled three times from pyrex glass stills fitted with a column 20 inches long to study the iron requirements of

bacteria. It is probably essential to distil water twice from pyrex glass when using micro-organisms or *Lemna* as a test organism for consistent results. Tauböck (1942) used a still made with a condenser of " rock crystal " to produce water for a test of aluminium requirements.

The heavy-metal content of water redistilled from pyrex glass is not easy to determine by chemical means owing to the need for purification of all reagents, concentration of a large volume of water to be tested and the similarity of the order of blank and actual determinations. Arndt and Hoffman (1937) detected ·0005-·001 p.p.m. Cu in twice glass-distilled water. Piper and Oertel (1941) and Piper (1942) reported not more than ·0002-·0004 p.p.m. Cu and ·001 p.p.m. total heavy metals in water obtained by single redistillation in pyrex glass. Millikan (1944) found that water similarly produced contained about ·001 p.p.m. of total heavy metals. Cliquet *et al.* (1933) reduced the conductivity of ordinary distilled water by a factor of 100 after distillation in a large (50-litre) pyrex glass still which had an output of 3 litres an hour. The equipment was, however, rather cumbersome. Hewitt and Hallas (1951) obtained *consistent marked* responses with *A. niger* to the addition of molybdenum at the rate of ·003 microgram per litre, by using metal-distilled water twice redistilled from pyrex glass stills, and found that water distilled once from the glass stills contained approximately 0·000003 p.p.m. Mo (Table 18). Vanselow and Datta (1949) concluded that such water contained less than ·000005 p.p.m. Mo. Boll and Street (1951) found that twice glass distilled water samples may vary significantly with respect to copper and molybdenum contents.

An interesting still is an all-glass steam stripping still* operated from live steam which is scrubbed by its own condensate and then collected after further movement of the vapour. This still should minimise metal contamination, especially that due to entrainment as spray, but the problem of possible " creep," if the steam is not clean or is oily, may still occur.

The early stills of Fitting (1928), Roberg (1931), Cliquet *et al.* (1933) were not designed for continuous operation, and do not merit further description. Those of Wilson and Strickler (1939), Holmes (1940), Liebig (1940), Piper and Oertel (1941), and Hewitt (Part Two) are intended for continuous operation with a minimum of attention. The stills designed by Piper and Oertel (1941), (Fig. 2) and those used at Long Ashton (Fig. 3) have supplied several thousand litres of high quality water and only need attention when refilling of the reservoirs is necessary. These stills are simple and rely on the principle of a Mariotte's bottle to maintain a constant level ; that described by Liebig (1940) used a ground-glass float valve.

The electrical efficiency of the all-glass electric immersion heaters, described by Piper and Oertel (1941), was about 90% and produced an output of 1·5 litres an hour with a power consumption of 1270 watts. The radiation electric pattern used at Long Ashton has an output of 0·65 litres an hour with an element consuming 600 watts and of 1 L. an hour with a 1,000 watt element, and therefore attains a similar but slightly lower efficiency. An automatic relay to cut out the current of the heater was recommended by Piper (private communication) to safeguard the effects of running dry. This can be arranged also to cut off the current when the receiving reservoir is filled. The relay recommended had platinum contacts and was controlled by a valve.

The output of the gas-heated stills may reach 2 litres an hour, but a rate of 1·3-1·6 litres an hour is recommended to reduce entrained spray. The condenser in the Long Ashton pattern still has a vestibule inside the condenser jacket so that condensation (and pressure reduction) may take place before the vapour comes into the reduced bore of the condenser tube proper. This is intended to maintain a high rate of distillation. The importance of a wide bore vapour path has been emphasised by Piper and Oertel (1941). The principal dimensions of the stills are given below :—

Capacity of boiling flask : 2-3 litres.
Diameter of splash traps : 60 mm.
Internal diameter of tubing and vapour bend : 18-20 mm.
Height of still overall : 50 cms. approx.

* Manufactured by Messrs. Townson & Mercer, Croydon, Surrey.

Joints between flask and splash trap B.34 Standard cone and socket.
Joints between vapour bend and condenser B.24 Standard cone and socket.
Length of vapour bend : 20 cms. approx.

The feed in the Piper and Oertel still is supplied from a 50-litre aspirator as a Mariotte's bottle; the level is entirely controlled by the position of the bottom end of the air inlet tube. The bung at the top must be completely air-tight. The aspirator is refilled by evacuating with a filter pump and connecting the supply inlet to a reservoir of distilled water. The tube connected to the boiling flask can be shut off with a stopcock and the outlet is designed to empty the boiling flask by siphoning for cleaning purposes.

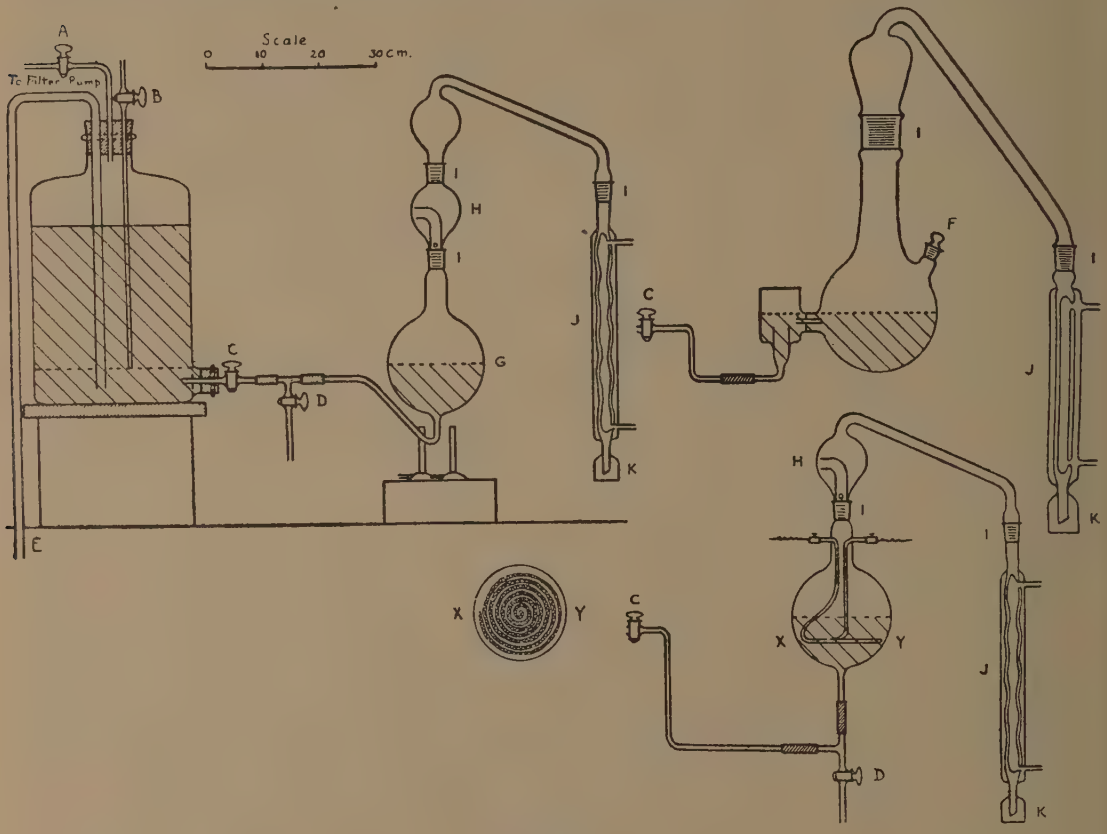


FIGURE 2

Constant-level pyrex glass stills (Piper and Oertel, 1941).

A = Stopcock to filter pump for refilling through tube E.
B = Stopcock and tube regulating air inlet to Mariotte's bottle and level of water in still.
C = Stopcock to isolate still.
D = Stopcock for flushing and draining still.
E = Large tube (11 mm. in diameter).
F = Side tubulure on silica flask.
G = Constant level still with basal inlet.
H = Splash trap.
I = Ground glass joints.
J = Double surface condenser (for rapid condensation).
K = Dust cover.
x-y = Coiled electrical heating element inside pyrex glass tubing for electrically-heated model.

PLATE E

General view of constant-level glass stills.

[*To face page* 56

PLATE F

The effect of water supply on the production of copper deficiency in tomato in sand culture.

Extreme left : **Control plants given ·065 p.p.m. copper. Remainder grown without additional copper, from left to right as follows :**

water from metal stills with pyrex glass condensers ;
demineralised rain water ;
once glass distilled water ;
twice glass distilled water.

Comparable results were obtained in a zinc deficiency trial.

A point that may be mentioned in connection with continuous-operating stills is the fact that whatever mineral contamination is present in the feed water is continually concentrated and the effects of spray are therefore likely to become progressively more serious with time. For this reason periodical cleaning may be advantageous even when the boiling flask and its contents still *appear* clean. Boll and Street (1951) showed that the zinc concentration in once glass distilled water fell from ·02 to ·001 p.p.m. after cleaning.

Demineralised Water

The first suggestion that synthetic resin ion-exchange methods might be applied to the purification of water for nutritional investigations was made by Liebig *et al.* (1943 a). These workers did not attempt to produce any deficiency effects, although the possibilities were mentioned, but showed that the effluent from Amberlite resins was non-toxic to citrus plants in water culture. These findings were confirmed by Hewitt (1946 a, c), (1947 a), for tomato, potato, sugar beet, cauliflower and other *Brassicæ*, beans, pea, clovers, flax, cereals and several other crops grown in sand culture. Arnon and Grossenbacher (1947) showed that tomato and lettuce plants actually *grown in contact* with the resins in sand culture were not injured by any toxic constituents. The suitability of such demineralised water for pharmaceutical purposes was demonstrated by Harrison *et al.* (1943). Demineralised water was also used by Curtis (1949 a), and in large-scale fruit cultures by Kenworthy (private communication). Loustalot *et al.* (1950) used demineralised water (treated by Amberlite resins) for preparation of nutrient solutions for tung seedlings in sand cultures.

Water purified by demineralisation was first used for deficiency experiments by Hewitt (1946 a, c) when Permutit synthetic resins were used to purify hard tap water and rain water. Deficiencies of nitrogen, phosphorus, potassium, calcium, magnesium and boron have been produced in tomato, kale, cauliflower, sugar beet and other plants with demineralised tap water, but Schroeder *et al.* (1946) did not succeed in producing boron deficiency in beet when demineralised tap water was used. Severe deficiency effects due to lack of the macronutrients and of boron, iron and manganese have been produced in a wide range of crop plants with demineralised rain water. The equipment now in routine use at Long Ashton produces an effluent that frequently compares quite well with glass distilled water as judged by the dithizone test of Stout and Arnon (1939) and may be markedly superior to " ordinary distilled water." Further treatment in large pyrex glass columns holding special resins, e.g. Biodemin-rolit F or IMAC C 11, produces water suitable for studies of copper, zinc and molybdenum deficiencies.

Demineralisation of rain water is ideally suited to the operation of large-scale culture experiments since several thousands of gallons of water comparable to distilled water are thus obtainable. The equipment and procedure recommended for treatment of rain water and tap water are described in Part Two. The purification of tap water by synthetic resins is useful when rain water supplies fail or as an alternative source of large-scale supply.

The quality of water produced by demineralisation may be judged from the analytical data given here and in Table 14. Liebig *et al.* (1943 a) found that ordinary distilled water was further purified when passed through Amberlite I.R. 100. The following results were obtained :—

Liebig *et al.* (1943 a)

	p.p.m.		
	Cu	Zn	Pb
Untreated distilled water	·2	·02	·055
Demineralised water, single passage	·0035	Nil	·0015
Demineralised water, five passages	Nil	Nil	·001
Distilled water redistilled from pyrex glass ...	·0016	Nil	·0025

The water passed through the resin five times was thus apparently superior to water redistilled once from glass.

FIGURE 3A *(right)*

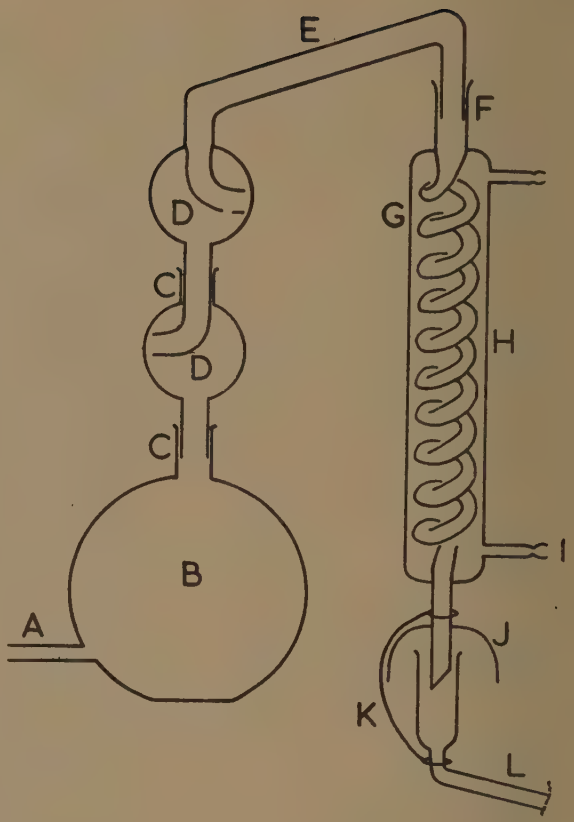
Pyrex glass constant-level still used at Long Ashton. Gas-heated model.

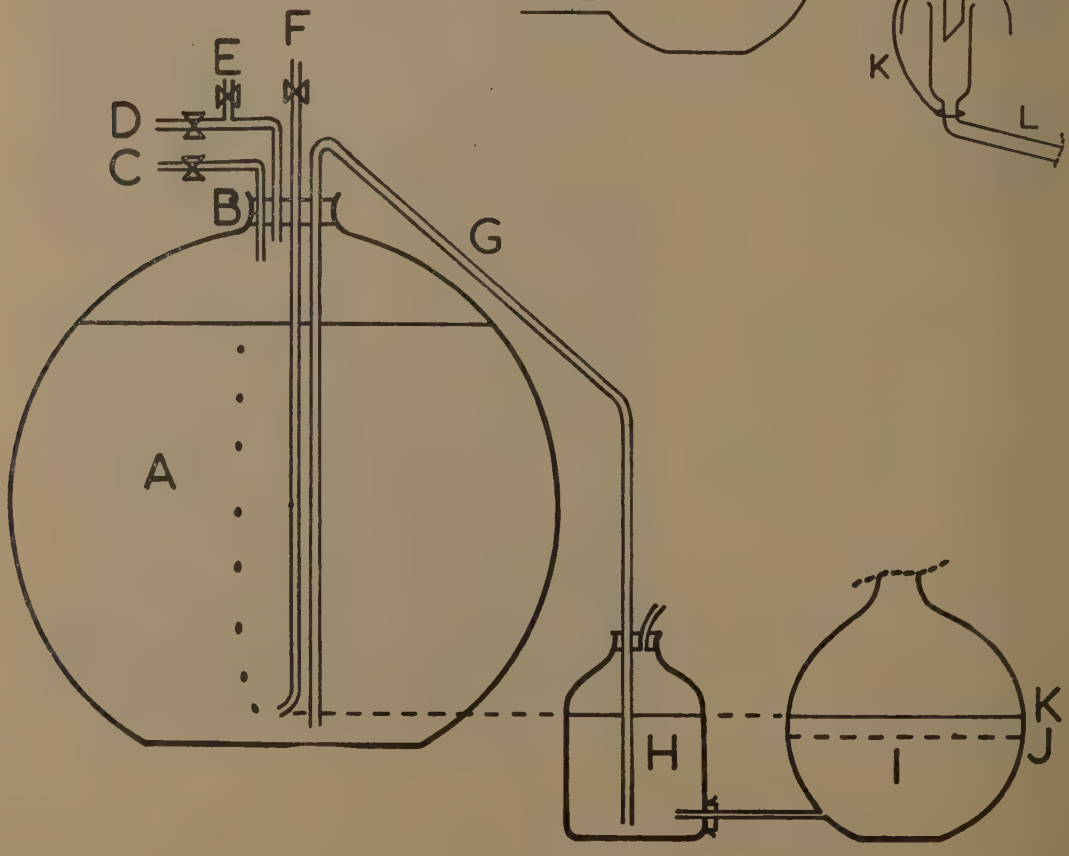
A = Inlet from constant-level reservoir.
B = 3-litre flask, output 1·5 l/hr.
C = B 34 joints.
D = Spray traps.
E = Wide bore vapour bends (15 mm. bore).
F = B. 24 joint.
G = Wide bore extended into condenser.
H = Condenser.
I = Water inlet.
J = Dust cover.
K = Supporting loop of string.
L = Delivery tube to carboys.

FIGURE 3B *(below)*

Pyrex glass constant-level still used at Long Ashton. Method used to maintain constant level feed to stills.

A = 50-litre reservoir.
B = Air-tight bung.
C = Refill inlet.
D = Attachment to suction pump.
E = Pressure release.
F = Air inlet and constant-level control tube with base turned slightly to side.
G = Siphon with stopcock. (Stopcock not shown).
H = 2-litre stabilizing vessel with air outlet.
I = Base of still.
J = Level when boiling.
K = Level when cold.





Liebig *et al.* (1943 a) also compared the mineral content of unpurified and demineralised tap water after a single passage. The following data show the results obtained in this experiment. The analyses were made spectrographically :—

Liebig *et al.* (1943 a)

p.p.m.	SiO_2	Ca	Mg	K	Na	B	Mo	V	Fe	Mn	Cu	Zn
Tap water	24	48	6	3	24	·1	·01	·1	·05	·001	·008	N.D.
Demineralised water	22·8	·01	·5	N.D.†	N.D.	·08	N.D.	N.D.	·002	·002	·002	·02

†N.D.=Not detected.

The great reduction in levels of calcium, potassium, sodium and iron are noteworthy. Boron was not appreciably reduced. The failure to remove much boron is confirmed by the results of Schroeder *et al.* (1946), and analyses in Table 14 also indicate a lower efficiency for boron removal than for many metal ions, although tap water so treated may be used to obtain boron deficiency effects in sugar beet and cauliflower as shown by Hewitt (1946 c). The lower efficiency of removal shown for boron and also molybdenum (Table 14) is due to the fact that the corresponding acids are relatively weakly dissociated and under these circumstances ions are less efficiently removed. New resin exchange materials are now available for dealing more efficiently with the less readily removable ions such as H_2BO_3', MoO''_4 and also silica.* Unpublished work by Hewitt (1950) (Table 18) indicates that suitable resins may be able to remove 99% and probably more of the molybdenum present, whilst the usual efficiency is 60-70% with " DeAcidite " E. The removal of iron from rain water (Table 14) is efficient, as the iron is present, presumably, mainly in the ionised *ferric* state ; removal of iron from hard tap water at Long Ashton, however, is not so effective (Table 14), as the iron is present mainly in the undissociated state as *ferrous* bicarbonate. Liebig *et al.* (1943 a), however, recorded efficient removal of iron from tap water.

The efficiency of removal of ions by demineralisation depends on several factors. Detailed discussion of the physico-chemical properties of these materials is beyond the scope of this work. Further details may be obtained in the original publication of Adams and Holmes (1935), and in later papers by Myers *et al.* (1941), where further references are cited. It is however, relevant to summarise a few important points that help to explain the behaviour of demineralising materials and the conditions most advantageous for their operation.

* The Permutit Co. " De Acidite " F and " Bio Deminrolit."

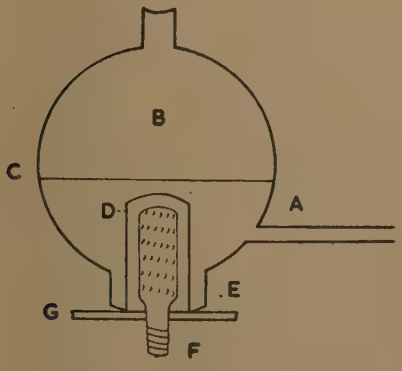


FIGURE 3c

Pyrex glass constant-level still used at Long Ashton. Section of flask for electrically-heated model.

A = Inlet from constant-level stabilizing vessel (H in Fig. 3b).
B = 3-litre flask adapted for electric heating.
C = Level of water during use.
D = Dome enclosing heater.
E = Extension of flask and dome.
F = Electric bowl fire element rewound for 1 kilowatt (output 1 l./hr.).
G = Asbestos board support.

TABLE 14

Data Showing Effect of Demineralisation on Removal of Ions from Rain Water and Tap Water at Long Ashton

A. Rain Water p.p.m.	Ca	Mg	K	Na	Fe	Mn	Cu	Zn	B	Mo
Untreated water	—	·14	—	—	·1	—	0·12	·2	—	—
Effluent from single cation and anion-removing units	—	·01	—	17	·02	—	·008	·03	—	—
Untreated water	1·04	—	—	—	·1	·016	·062	·44	·006	·00018*†
Effluent from two cation and one anion-removing units	·05	—	—	—	·003	·003	·006	·012	·0022	·000046*†
Comparison data for water from tinned copper stills :										
with tinned condensers	—	—	—	—	·004-·006	·004	·014	·0075	·003	—
with silver condensers	—	—	—	—	—	—	—	—	—	·00006*
B. Tap Water										
Untreated water	102	11·2	K+Na 11·0	—	·04	—	—	·07	—	—
Effluent from single cation and anion-removing units	1·2	0·1	—	18·0	·03	—	—	·035	—	—
Effluent from two cation and one anion-removing units	·15	·0074	K+Na ·35	—	·03	—	—	·022	—	—

* Results obtained by microbiological assay with *Aspergillus niger*.

† Further treatment may reduce the level to below ·00001 p.p.m.

The ion-exchange resins are of two types for cation and anion removal and are contained in separate units. The reaction involving the removal of cations by exchange for hydrogen ions on the Zeo-Carb or Amberlite IR 100 types is an equilibrium reaction and cannot therefore proceed to absolute completion ; a similar limitation applies to the anion-removing resins such as De Acidite or Amberlite IR 4. The equilibrium point is usually greatly in favour of the adsorbed state and values of over 90% adsorption are usual for divalent ions like those of calcium or magnesium. Ions of weakly dissociated salts, such as borate and molybdate, are less readily removed than those of the more highly dissociated ones. The removal of some ions is somewhat less efficient from more dilute solutions as indicated by the data in Table 15, where the relative efficiency of removal of some cations and anions is shown. In line with many types of exchange reaction, the efficiency of removal of ions increases with valency and the concentrations of mixtures of ions of different valency in the adsorbed and solution states may differ greatly, as shown by the work of Arnon and Meagher (1947) discussed in the Section on solid rooting media. The efficiency of removal is modified by the pH of the solution. The adsorption of cations is reduced by increase in hydrogen ion concentration and that of anions is increased. Organic complex-forming compounds may interfere with ion-exchange removal. Thus treatment of rain water may fall short of that expected and tests at Long Ashton suggest that removal of low concentrations of molybdenum from rain water may be less efficient than from distilled water.

TABLE 15

Examples of Relative Efficiency of Removal of Certain Ions from Water by Demineralisation

Initial Concentration p.p.m.	Effluent Concentration p.p.m.			% Efficiency		
	A.	B. After passage through 1 unit	C. After passage through 2 units	B/A	C/B	Overall A-C/A
Ca^{++}	102	1·2	0·15	98·8	87·5	99·85
Mg^{++}	11·2	0·1	0·0074	99·1	92·6	99·93
Fe^{++} (tap water)	·084	—	0·03	—	—	64·3
Fe^{+++} (rain water) ...	·093	—	0·003	—	—	97
Zn^{++} ,, ...	·2	0·03	—	85	—	—
,, ...	·44	—	0·012	—	—	98·7
B (as H_2BO_3') (tap water) ...	·07	0·035	—	50*	—	—
,, ...	·053	—	0·022	—	—	58·5
B (as H_2BO_3') (rain water) ...	·006	—	0·0022	—	—	63·3
Mo (as MoO_4'') (rain water) ...	·00018	0·000046	—	74*	—	—

* Treatment with De Acidite E.

In large industrial installations it is customary continuously to recirculate a part of the once-treated water and inject it into untreated water. This procedure further reduces the mineral content obtained with the use of a single unit. For small-scale installations designed to purify water for culture experiments a more suitable method is to arrange a number of smaller units in series so that cation- and anion-receiving units are placed alternately. Such an arrangement is shown in Part Two. This method has certain advantages. Each repetition of a particular unit not only operates at a progressively lower concentration and is therefore able to carry the equilibrium further towards completion, but also serves as a safeguard against " break through," should this occur unexpectedly due to exhaustion of the first unit in the series. The use of more than one cation-receiving unit is also useful to reduce the content of sodium in the effluent from the preceding anion-removing unit. The concentration of sodium tends to be high owing to the use of sodium hydroxide or sodium carbonate for regeneration. In spite of the fact that the units are rinsed after regeneration, sodium

tends to persist in the effluent, especially when relatively soft water such as rain water is used. It is convenient to complete the chain of alternating units with a cation-removing unit as this ensures a high quality effluent relatively less contaminated with the micronutrient cations, or to divide the effluent between two appropriate resins.

When the exchange units are saturated and further exchange no longer occurs the " break through " point is reached. This state occurs relatively suddenly and the resins may be used for exchange at practically maximum efficiency until nearly completely saturated. The saturated units have to be restored by " regeneration " to their initial exchange capacity by displacing the adsorbed ions. This is carried out by saturating the resins with a solution of an acid to supply hydrogen ions for regeneration of cation units or of an alkali to displace the adsorbed anions. The exchange reaction between hydrogen ions and other cations during demineralisation results in an acid effluent. The pH of the solution is characteristic of the water being treated and remains constant until " break through " occurs, at which point it starts to increase rapidly as shown in Figure 4. The pH of the effluent from the anion-removing unit, which is initially high, falls steadily after regeneration and thus behaves differently from that of the effluent from the cation-removing unit. The relative suddenness of the " break through " makes the development of a suitably sensitive test of the effluent a matter of some importance. The relation between " break through " for calcium or magnesium and pH shown in Figure 4 suggests that the use of pH as suggested by Kenworthy and Howard (1944) is not satisfactory as a test of " break through," since this may be an accomplished fact for magnesium before the change in pH is appreciable. The alternative of using conductivity of the effluent from the first pair of units as a measure of determining " break through " is probably the most sensitive physical method, but, in common with any chemical test for a particular ion, the test is indeterminate until almost too late to anticipate the onset of " break through." Conductivity, however, is an additive function and therefore relatively more sensitive than testing for change in the concentration of any one ion. A device such as that described by Blake (1945 a) for the indication of small changes in electrolytic resistance would probably be suitable for this purpose. The safest procedure probably is to determine experimentally the volume of water of a particular hardness that can be safely treated before " break through " occurs and to allow a reasonable safety margin of, say, 10%.

Comparison of the analyses for demineralised rain water and for water obtained from metal stills (Table 14) shows that the former is comparable and may be superior to the latter. The effluent from the chain of five ion exchange units in use at Long Ashton frequently passes a dithizone test for heavy metals similar to that devised by Stout and Arnon (1939). A routine check on the quality of the effluent is also obtained with the sensitive manganese test using tetramethyldiaminodiphenylmethane and potassium periodate, Wenger and Duckert (1941), Nicholas (1946). Details of the method are given in Part Two.

Activated carbon. The use of activated carbon is often adopted for the removal of taints from water. Iron in organic complex form is not readily removed by demineralisation methods according to Howard and Spooner (1946), but may be greatly reduced by treatment of water with activated carbon. Experiments by the writer have been made to determine the value of activated carbons for the removal of traces of micronutrient elements from rain water which naturally contains some organic matter. It was found that certain activated carbons were able to remove over 90% of the iron from solutions of ferric citrate containing ·1-10 p.p.m. iron, but removal of boron and manganese was poor.

In these experiments a carbon column was placed in series with the demineralisation equipment *following* the final cation-removing unit, as it was expected that a small column would have a maximum life in this position. It was found that under these conditions the somewhat acidic effluent (pH 3·5-4) appeared to release appreciable amounts of several metallic ions from the carbon, although removal of iron and copper from untreated rain water was demonstrated. The insertion of a carbon column prior to the ion-exchange units was not tested, but a large column might be advantageous in this position, or after an anion exchange. There is certainly scope for further investigation of the application of carbon as a means of removing specific contamination.

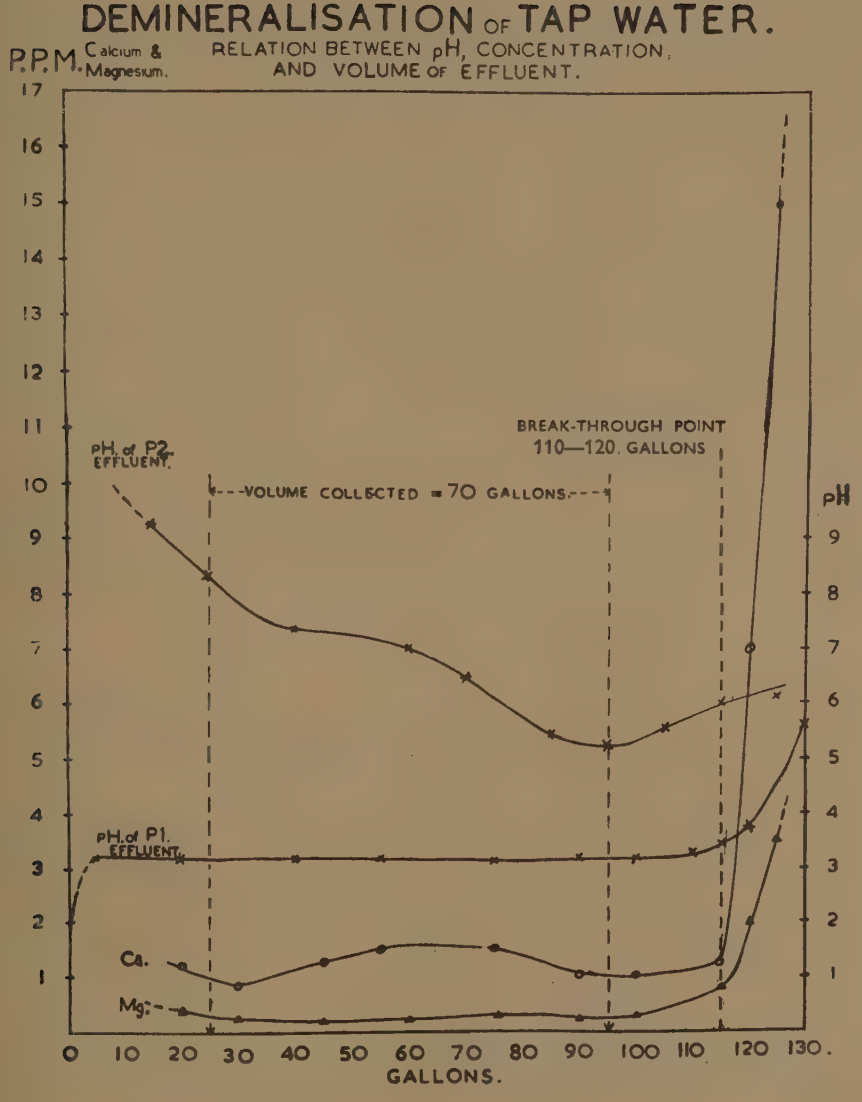

DEMINERALISATION OF TAP WATER.
P.P.M. Calcium & Magnesium.
RELATION BETWEEN pH, CONCENTRATION, AND VOLUME OF EFFLUENT.
BREAK-THROUGH POINT
110—120. GALLONS
pH of P2 EFFLUENT.
---VOLUME COLLECTED = 70 GALLONS---
pH of P1. EFFLUENT.
Ca.
Mg.
pH
GALLONS.

FIGURE 4

NUTRIENT IMPURITIES AND PURIFICATION METHODS

The purity of reagents used for the preparation of nutrient solutions is a matter of great importance when micronutrient deficiencies are investigated. Those used for nutrient solutions in macronutrient studies do not usually require further purification provided that salts of " recrystallised " or " laboratory grade " are used. Nutrient solutions prepared from materials of this quality may, however, contain up to 0·2 p.p.m. of calcium (Hewitt unpublished), and this may possibly delay the onset of acute calcium deficiency in some plants. These grades are free from toxic concentrations of heavy metal impurities. Materials of lower grades, as for example " commercial " or " fertiliser " grade, may well contain injurious amounts of boron, zinc, fluoride or other constituents, cf. for example, Cutts (1935), Young (1935), Gaddum and Rogers (1936), Rader and Hill (1938), Ayers and Hatcher (1939), Stout and Arnon (1939), Lehr (1940), Walkley (1940), Gray (1944), Brenchley (1946), and cannot thus be generally recommended unless tested individually.

The nature and concentration of the impurities in the reagents commonly used for preparing nutrient solutions vary widely and the purest grades available commercially (for example analytical reagent and batch analysed grades) are frequently still too greatly contaminated by micronutrient elements, for example molybdenum noted by Bertrand (1940), Mitchell and Scott (1948), to permit their use successfully without further treatment. Some analytical reagent grade salts may often be more contaminated than less refined commercial grades according to Ayers and Hatcher (1939), Stout and Arnon (1939), Walkley (1940). Whilst the principle of routine purification is justified in most instances where the study of minute concentrations and deficiencies of micronutrients is concerned, it is helpful to examine the nature and distribution of the probable impurities in various grades of nutrient reagent salts and the raw materials from which some of them may be obtained. Instances where these reagents have been used successfully without further treatment also merit attention. Information on these points may be of use in indicating where extra precautions are advisable or where one salt may be replaced by another less likely to be contaminated, by a particular element.

The Impurities in Nutrient Salts and Raw Materials

Phosphates

Phosphate materials, both natural and processed, appear to be often heavily contaminated, but the nature and degree of contamination varies greatly. Rader and Hill (1938) examined the boron content of several phosphate samples and found considerable variation according to source. Apatite and defluorinated rock phosphates had a low boron content of about 10-20 p.p.m. as B_2O_3. South Carolina phosphate contained about 130 p.p.m., African phosphate contained 66-116 p.p.m., and " inland " phosphates 10-72 p.p.m. They considered that phosphoric acid prepared from these sources would contain a large proportion of total boron. Hewitt (unpublished) found that recrystallised grade sodium dihydrogen phosphate was generally low in boron (0·5 p.p.m. or less), but a sample of the anhydrous compound contained 7 p.p.m. This contamination may have arisen from the pans during the process of dehydration as indicated by Rader and Hill (1938) for anhydrous calcium chloride. Dipotassium phosphate was found by Hewitt (unpublished) to contain 11 p.p.m. of boron. Lehr (1940) examined several phosphates for boron and found the usual contamination was only 1-2 p.p.m.

Phosphate materials may be heavily contaminated by zinc. Walkley (1940) found that island rock phosphates contained from 600 to 1,000 p.p.m. of zinc, 75-110 p.p.m. cadmium and less than 50 p.p.m. of copper and manganese. Superphosphates contained up to 600 p.p.m. of zinc, and Ayers and Hatcher (1939) found potassium dihydrogen phosphate to contain 35-200 p.p.m. of zinc. The data of Gray (1944) indicate comparatively little zinc and copper in natural phosphates but relatively high concentrations (25-500 p.p.m.) of manganese and iron. Young (1935) examined a variety of natural and manufactured phosphate materials and concluded that all were usually high in manganese and might contain up to 0·16%. The copper content was usually 80 p.p.m. or less. The boron content ranged from 0-300 p.p.m. and was apparently not related to source as found by Rader and Hill (1938). Gaddum and

Rogers (1936) found that, compared with other fertiliser materials, phosphates were generally heavily contaminated with a wide range of elements. They detected up to 0·5% each of manganese, vanadium and boron, and from 50 to 1,000 p.p.m. of nickel, cobalt, copper and zinc. Gray (1944) found that phosphates may contain up to 2 p.p.m. of molybdenum. Hewitt and Hallas (1951) showed that sodium phosphate, even after repeated recrystallisation, was still relatively heavily contaminated by molybdenum (0·03 p.p.m.) compared with similarly treated magnesium sulphate and potassium nitrate (see Table 18). Mulder (1939 a) found that dipotassium phosphate was especially likely to be contaminated by molybdenum, but Vanselow and Datta (1949) found samples of potassium phosphate spectroscopically free from molybdenum. Baird (private communication) reported that spectrographic tests on several samples of ferrous phosphate revealed 30-60 p.p.m. molybdenum.

Potassium Salts

In contrast to phosphates, potassium compounds are frequently relatively pure, as shown by Young (1935), Gaddum and Rogers (1936) and Walkley (1940). Potassium salts, especially potassium nitrate, are often low in manganese, zinc and other metals, but may contain variable amounts of boron, according to source. The synthetic product may be practically devoid of boron whilst the natural material may contain up to ·07%, Young (1935). Ayers and Hatcher (1939) found that, whereas the " C.P." grade of potassium chloride contained no detectable amount of boron, the technical grade contained 10-170 p.p.m. Technical grade potassium nitrate only contained 1·5 p.p.m. of boron but might contain between 0·5 and 20 p.p.m. of zinc compared with only 1 p.p.m. in the chloride. Walkley (1940) also found that potassium chloride from France, Germany or Palestine contained less than 1 p.p.m. of zinc. Stout and Arnon (1939) record that a reagent grade sample of potassium nitrate was heavily contaminated by 100 p.p.m. zinc compared with only 0·1 p.p.m. in a fertiliser grade sample. Hewitt (1947, unpublished data) found that a sample of analytical reagent grade potassium sulphate contained 9-13 p.p.m. boron, negligible amounts of manganese and up to 0·6 p.p.m. of molybdenum ; potassium nitrate of comparable grade contained 5 p.p.m. boron and ·05 p.p.m. manganese, and only ·003 p.p.m. molybdenum.

Nitrates

Nitrates vary widely in purity mainly according to their origin. Thus Chilean nitrate of soda is notable for its numerous micronutrient and other impurities, as was shown by Cutts (1935), Shive (1938), Brenchley (1946), Warington (1946), whereas Young (1935) and Lehr (1940) found that synthetic nitrates are often relatively pure. Synthetic and natural nitrates appear to be relatively free from zinc, copper and manganese according to Young (1935), and Walkley (1942). The freedom of nitrates from manganese has also been confirmed by the writer, but appreciable quantities of boron were detected in many nitrates as follows :— magnesium nitrate 12 p.p.m., potassium nitrate 5 p.p.m., calcium nitrate (anhydrous) 4 p.p.m., calcium nitrate prepared from nitric acid (redistilled) and calcium carbonate 0·7 p.p.m.

The molybdenum content of nitrates may vary and anhydrous calcium nitrate may be relatively heavily contaminated, possibly during dehydration according to Steinberg (1937), (1939) (Table 16). Warington (1946) reported that Chilean nitrate also contained an appreciable amount of molybdenum. Hewitt (1947 unpublished) found about 0·5 p.p.m. molybdenum in samples of magnesium nitrate (recrystallised grade) and anhydrous calcium nitrate compared with only 0·005-0·15 p.p.m. in sodium and potassium nitrates (analytical reagent grade). Vanselow and Datta (1949) found molybdenum in all samples of magnesium and calcium nitrate but not in potassium nitrate.

Other Salts

Magnesium sulphate may be appreciably contaminated by several micronutrients. Thus Hewitt (unpublished) found that a sample of recrystallised grade salt contained 24 p.p.m. manganese, 25 p.p.m. boron and 0·3 p.p.m. molybdenum, and samples of analytical reagent quality contained 0·4-0·6 p.p.m., 9-10 p.p.m. and 0·15 p.p.m. respectively. Ayers and Hatcher

(1939), on the other hand, detected only 1 p.p.m. of boron in a sample of technical grade magnesium sulphate and Lehr (1940) could not detect boron in magnesium sulphate. Manganese contamination was noted by Piper (1944) and iron, copper and manganese by Yoshimura (1934). Steinberg (1936) (1937) regarded magnesium sulphate as a main source of contamination by molybdenum in cultures of *A. niger*, and great improvement was obtained by substituting the compound by magnesium nitrate and sodium sulphate (see Table 16), contrary to the findings of Vanselow and Datta (1949). The magnesium sulphate also yielded appreciable amounts of iron and zinc. Chapman *et al.* (1937) found 8 p.p.m. of zinc in pure magnesium sulphate used for zinc deficiency work. Hewitt and Hallas (1951) found that thrice recrystallised analytical reagent grade magnesium sulphate contained 0·01-·026 p.p.m. molybdenum as determined by the growth of *A. niger* in standard purified culture solutions. Evans *et al.* (1951) found 2-4 p.p.m. molybdenum in samples of fertiliser calcium carbonate.

Ammonium sulphate and sulphuric acid may vary greatly in the degree of their contamination by zinc. Walkley (1940) noted that lead chamber products might contain up to 620 p.p.m. zinc, whilst contact acid derivatives contained only 2-7 p.p.m. Ayers and Hatcher (1939) found 5-20 p.p.m. zinc in ammonium sulphate. Beckwith (private communication) found 0·02-0·06 p.p.m. molybdenum in samples of ammonium sulphate. Yoshimura (1934) found that ammonium salts were notably contaminated by copper. Boll and Street (1951) concluded that the macroelement salts used to prepare root culture media contributed ·1, ·01 and ·0001 p.p.m. or less respectively of manganese, copper and molybdenum.

Iron and Heavy Metal Salts

The micronutrient salts and iron compounds used in the preparation of culture solutions are generally relatively heavily contaminated with certain heavy metals, notably copper, zinc, lead, manganese and molybdenum. The amounts commonly used (with the exception of iron salts) do not, however, appear to justify the application of elaborate purification procedures, except when toxic concentrations of one is to be given in the presence of deficiency levels of another, or where micronutrient deficiencies in *A. niger* cultures are concerned, or if the possibility of hitherto unrecognized micronutrients is to be tested.

Iron compounds, however, are given in concentrations sufficiently high to make the actual nature of the probable impurities more important. The presence of boron does not seem to have been recorded and is not apparently significant. Iron compounds are often carriers of manganese as noted by Bertrand and Javillier (1911), (1912 a, b), Samuel and Piper (1929), Haas (1932 b), Löhnis (1944), Piper (1944), Hewitt (1947 unpublished), Twyman (1945 unpublished), who found ·046% manganese in ferrous sulphate analytical reagent grade, and molybdenum, according to Horner and Burk (1934), Piper (1944), Hewitt (1947 unpublished) and Evans *et al.* (1950), who found 0·1 p.p.m. molybdenum in samples tested. Zinc and copper may occur as in the instances noted by Chapman *et al.* (1937) and Piper (1944) ; ferrous sulphate may contain 20 p.p.m. zinc. The significance of cobalt contamination of iron salts was noted by Hutner *et al.* (1950), who found that spectroscopically pure iron salts yielded adequate cobalt for the formation of vitamin B_{12} by *Euglena*.

Experiments performed using Unpurified Salts

The possible undesirable effects of *heavy* contamination by boron, zinc, manganese and other elements of the order of 0·1% to 1% in the salts used for the preparation of nutrient solutions needs little further comment, when it is realised that a nutrient solution thus prepared will contain from 0·1 to 1·0 p.p.m. of impurity for every 100 p.p.m. of each nutrient salt. The presence of much lower levels of contamination, however, are frequently sufficient to prevent the production of deficiency symptoms when the effects of lack of copper, zinc, molybdenum and even of iron, manganese or boron are under test. There are, however, many instances where successful results have been obtained by the use of unpurified salts.

Boron deficiency, particularly in susceptible plants like broad bean, beet, tomato, is probably the most widely demonstrated micronutrient deficiency produced with the use of commercially available salts without further treatment. This is shown, for example, by the

work of Brenchley (private communication), Warington (1923), Brenchley and Warington (1927), at Rothamsted, and by Hoagland and Snyder (1933), Hill and Grant (1935), Eltinge (1936), Löhnis (1937), Shive (1938), Lehr (1940). In the Rothamsted work, however, a routine spectrographic examination was made and large batches selected when a satisfactory sample was obtained. Lehr (1940) used a chemical method to detect boron.

Manganese deficiency has been produced in several relatively susceptible crops such as tomato, beans and maize, by Bishop (1928) who used Merk " pro analysi " grade, by Miller (1928) who used Mallenkrodt grade salts, by Samuel and Piper (1939) with analytical reagent grade salts tested by the periodate method and by Shive (1938) using C.P. grade salts. Zinc deficiency, and less effectively, copper deficiency also were produced in tomato by Stout and Arnon (1939). Zinc deficiency symptoms were observed in citrus by Chapman *et al.* (1937), in spite of detection of traces of zinc in some of the reagents, including 3 p.p.m. in the magnesium sulphate used. The initial contamination of 0·007 p.p.m. zinc in solution was reduced to 0·003 p.p.m. after 3 weeks' growth.

Molybdenum deficiency was observed in lettuce (but not reproducibly) by Warington (1946) who used unpurified analytical reagent grade salts, and by Arnon and Stout (1939 b) and Piper (1940) in experiments where the purification procedure is now considered not to have been specifically effective for the removal of that element (cf. Section on nutrient purification). That commercially available salts can be obtained almost free from molybdenum is indicated from the work of Steinberg (1939) (1945 a) in which yields of *A. niger* grown without molybdenum can be reduced to 10% or less of the controls by choice of suitably pure samples. Mulder (1948 a) in fact used this response of *A. niger* as a proof of the purity of the salts to be used in molybdenum deficiency experiments. Thiman and Edmondson (1949) produced marked reduction in growth of *Polyrhiza* when manganese, zinc or molybdenum were omitted from a nutrient prepared with C.P. grade salts.

There are also numerous examples of the need for further purification of nutrient reagents used in micronutrient deficiency work. The early work of Mazé (1919) showed that the commercially obtainable " pure " salts allowed normal growth of maize in the absence of boron whilst salts especially purified for him caused marked reduction in yields, which were largely corrected by addition of boron. Sideris *et al.* (1943) noted that, although iron deficiency in pineapple could be observed in the presence of nitrate, the C.P. grade salts supplied sufficient iron when ammonium nitrogen was used. Hopkins (1934) in a study of manganese deficiency in *Lemna* found great variation in yields due to the use of nutrients prepared from two different batches of reagents. Stout and Arnon (1939) found that manganese deficiency in tomato was only produced by the use of purified salts and that although copper and zinc deficiencies appeared when unpurified salts were used, the effects were enhanced after purification. Roberg (1931) found that the growth of *A. niger* in unpurified culture media was equal to that obtained after the addition of iron, copper and zinc to purified media. The principal impurity was found to be zinc. Purification reduced the yields from about 1,300 mg. to 10 mg. Similar results were obtained by Gollmick (1935). Steinberg (1919), (1937), traced the stimulation of growth by *A. niger* in artificial media to the presence of zinc and other impurities in the magnesium sulphate. In one experiment, however (Steinberg 1938 b), spectrographically pure salts gave lower yields with zinc deficiency than when they had been purified, but opposite effects occurred for copper, iron and manganese.

Bertrand (1940) failed to produce molybdenum deficiency in peas in cultures where the nutrient salts yielded 0·004 p.p.m. of molybdenum. Steinberg (1936) found that molybdenum deficiency in *A. niger* was difficult to induce in the presence of magnesium sulphate owing to the frequent presence of molybdenum as an impurity, and a greater response to omission of iron or zinc was also obtained after purification. Steinberg (1939) also found that molybdenum deficiency depended greatly on the source of the calcium nitrate used and yields, where molybdenum was omitted, varied from 10% to 97% of the complete nutrient cultures according to the sample of calcium nitrate. Hewitt and Hallas (1951) found that no molybdenum deficiency effects were produced with *A. niger* if unpurified salts were used, and that the yield was only reduced by about 5% compared with 95% reduction after purification.

Boll and Street (1951) recorded a similar result when unpurified salts were used for tomato root tissue cultures owing to the unsuspected presence of copper and molybdenum in particular batches of reagents according to their origin.

With regard to the use of *A. niger* as a test organism or for other purposes it is necessary to note the presence of micronutrient impurities in the sugars used. These were found to be the main source of contamination by iron, zinc and copper by Roberg (1931), and significant by Steinberg (1936) (1937) (1938 a) and by Horner *et al.* (1942) in the study of molybdenum deficiency, where they introduced up to 4 μg molybdenum per culture. The production of responses to gallium by *A. niger* and *Lemna* in experiments by Steinberg (1938 b) (1945 b) could not be repeated after exhaustion of a particular batch of sugar (cf. Steinberg (1945 a)). Glasstone (1947) also noted the likelihood of impurities in sugar solutions prepared for the study of micronutrients essential for excised root cultures. Young *et al.* (1944) failed to obtain any response to omission of copper, manganese, zinc or molybdenum with *Escheria coli* in spite of attempts to purify the media.

Methods of testing Nutrient Purity

The data presented here on the micronutrients in nutrient reagents show that many elements are present in quantities that can be detected by chemical or physical methods. The application of such tests can therefore be used to eliminate heavily contaminated materials. The spectrographic method is useful inasmuch as it permits of a rapid and extensive survey of the impurities, and was used by Chapman *et al.* (1937), Steinberg (1937), Shive (1938), and Brenchley (private communication). The quantitative interpretation is, however, less secure when solid, relatively pure, salts are examined and a negative response can only be regarded as indicating a concentration less than a certain limit, which may still be appreciable. For example, the limit of detection of boron in solid magnesium sulphate is about 5 p.p.m., whereas the chemical methods based on distillation of the methyl borate ester are sensitive to 1 p.p.m. or less.

Spectrographic tests were used by Steinberg (1937 (1939) for selection of salts for use with *A. niger* cultures. Spectrographic freedom from zinc appeared to be a reliable guide to the selection of satisfactory reagents. A similar conclusion seems justified from the work of Chapman *et al.* (1937), who obtained positive results for zinc deficiency in citrus in spite of the identification of " low " concentrations of zinc in some of the nutrient reagents. On the other hand, it is generally agreed that a spectrographically negative response for molybdenum is not sufficient to ensure molybdenum deficiency. Thus Steinberg (1939) emphasised that the spectrograph is insufficiently sensitive to detect amounts of molybdenum that will permit normal growth of *A. niger*. The critical limit for normal growth appears to be about 0·005 μg per 50 ml. culture. This corresponds to 0·1 μg molybdenum per litre and to between ·01 and ·05 p.p.m. in any of the solid nutrient salts according to their concentration in solution. For work with molybdenum deficiency the biological test using *A. niger* as described by Mulder (1948 b), Hewitt and Hallas (1951) and Nicholas and Fielding (1951) is therefore to be preferred. Hopkins (1934) also stressed the value of a biological test for nutrient purity. Vanselow and Datta (1949) increased the sensitivity of spectrographic detection of molybdenum by prior extraction with stannous chloride, thiocyanate and ether. Boll and Street (1951) reported spectrographic evidence of several microelements in reagent grade salts. Separate batches varied considerably.

The results given for boron and zinc by Ayers and Hatcher (1939) and by Lehr (1940) were obtained from chemical tests and some idea of the limit of sensitivity obtainable thereby may be gained from the lowest positive figures of less than 1 p.p.m. for boron and ·05 p.p.m. for zinc. The sensitive procedure used to detect boron at low concentrations is described in Part Two. The diphenylthiocarbazone test for total heavy metals recommended by Stout and Arnon (1939) has a limit of sensitivity of about 0·001 mg/litre and possibly even greater sensitivity under carefully standardised conditions. Reagents or nutrient solutions that pass through this test are satisfactory for the production of acute zinc or copper deficiency in higher plants according to Stout and Arnon (1939). The tetramethyldiaminodiphenyl-

methane and potassium periodate test for manganese recommended by Wenger and Duckert (1941) and modified by Nicholas (1946) has a comparable sensitivity and is used to test the suitability of nutrient solutions for manganese deficiency as described in Part Two.

It may be concluded that spectroscopic or chemical tests may often be satisfactory for the examination of reagents with respect to zinc, copper, manganese and boron, but not molybdenum. The inclusion of a radio-active isotope of a micronutrient in a solution in order to test the quantitative efficiency of removal by a purification method should be a sensitive method of test, but does not appear to have been used so far as the writer is aware.

Conclusions

This survey of the nature and extent of micronutrient element contamination in the technical and purer grades of commonly used nutrient salts leads to the following conclusions :—

(1) There is no guarantee that any grade of reagent, including the purest available commercially, is even reasonably free from one or more of the known micronutrients.

(2) Naturally-occurring or fertiliser grade materials such as nitrates may contain lower concentrations of zinc and other elements than some analytical reagent grade products, but synthetic nitrates are generally the least contaminated.

(3) Phosphates are usually relatively heavily contaminated with several elements compared with nitrates and potassium salts. Magnesium compounds appear to occupy an intermediate position.

(4) It is occasionally possible to obtain batches of each main nutrient salt sufficiently low in micronutrient impurities to permit the production of deficiency symptoms without special purification.

(5) Spectrographic or chemical methods to test for the presence or absence of various elements are useful to indicate contamination by copper, zinc, manganese or boron, but may not be sufficiently sensitive to ensure that molybdenum deficiency can be expected by the use of " spectroscopically pure " materials.

(6) The numerous examples of the improvements in micronutrient deficiency technique gained by the application of an *appropriate* method of purification justifies the routine use of such methods in experiments of this nature, even when supposedly pure reagents are employed, and ensures consistent results.

Methods used for the Purification of Nutrient Reagents

The rigorous standards of purity needed to ensure the production of severe micronutrient deficiency effects in higher plants or microorganisms often require the application of specialised methods of purification. The difficulties inherent in attempts to remove traces of unwanted elements are often great. The usual methods of purification such as recrystallisation may be ineffective, especially for the removal of the " heavy metals." The reasons for the failure of such methods may lie in the effects of crystal isomorphism, ionic radius, occlusion, surface adsorption and other phenomena underlying the geochemical principles of Goldschmidt (1937). These principles also explain the nature of some of the common impurities in nutrient salts. Additional to these difficulties is that of handling highly or sparingly soluble salts such as calcium nitrate or calcium sulphate. Effective methods must also conform to other requirements, including (a) the avoidance of the use of toxic substances in the form of either solvents or chelation or precipitation reagents which cannot be readily removed, (b) reasonable simplicity and suitability for large-scale application, (c) reproducibility.

Methods which have been used for various purposes and which are reviewed and compared here include the following :—

Recrystallisation or precipitation (also continuous extraction of solid reagents and distillation of acids, etc.).

Adsorption on charcoals.

Adsorption on alkaline earths, carbonates or phosphates.

Co-precipitation by organic reagents, sulphides, etc.
Extraction by immiscible solvents, especially after chelation.
Other methods.

The use of purified salts by Salm-Horstmar (1851) is of some historical interest. Attention was given to the preparation of calcium nitrate free from magnesium and chlorine and of ferric oxide (Fe_3O_4) free from manganese. In the use of the methods described below, the possibility of the reintroduction of an impurity must always be recognized. The use of filter papers requires especial consideration as pointed out by Glasstone (1947). Experience at Long Ashton suggests that Whatman No. 42 papers are satisfactory for filtration of solutions purified for molybdenum and other micronutrient deficiencies, but the reintroduction of minute amounts of molybdenum has been demonstrated. Some investigators have syphoned off or decanted the supernatant solution after a precipitation, but this method is unreliable. The use of No. 3 or No. 4 porosity pyrex glass sintered filters is probably the most reliable method and least likely to introduce additional contamination, but cleaning afterwards may present difficulties.

Recrystallisation

Some of the limitations in the use of recrystallisation have already been outlined above. Hughes (1943) in a detailed study of preparative methods for obtaining pure compounds for spectrographic standards states in a concluding paragraph : " Recrystallisation methods are to be recommended only as a last resort, frequently these methods have been found ineffective, expensive . . . and wasteful . . . careful consideration must be given to the various factors involved in the separation, the relative atomic radii and ionic potentials of the various elements, compactness of anion involved, solvent composition and pH, solubility of the major constituent in the proposed solvent, and exact conditions under which crystallisation is effected." Hughes described elaborate procedures for the preparation of magnesium salts and calcium sulphate free from barium and strontium. Recrystallisation is the most effective means for this purpose but the purified salts still require treatment by other means to ensure freedom from the more common micronutrient metals.

Archibald (1932) considered that potassium nitrate was the most suitable potassium salt for purification by recrystallisation. He considered that recrystallisation was almost useless if the impurities were isomorphous with the principal salt, and pointed out the particular effectiveness of centrifugal means to remove the last traces of mother liquor from the crystals. This greatly enhanced the efficiency of purification, especially as more numerous washings with greatly reduced volumes of solvent could be employed.

Recrystallisation seems to be a satisfactory method for the elimination of boron impurities. Salts such as magnesium sulphate, potassium nitrate and sodium phosphate can be obtained with consistently less than 1 p.p.m. of boron, Hewitt (1946 a) (1947 a) (and unpublished), by two or three recrystallisations. Overall yields of 80-90% may be obtained by the addition of alcohol during cooling as described in Part Two ; this method was also employed by Roberg (1928). Sommer and Sorokin (1928) used salts recrystallised several times in a study of the effects of boron deficiency on the root tip of *Pisum sativum*.

Numerous investigators have used recrystallised salts in studies of copper deficiency. In the first demonstration Sommer (1931) used salts treated thus and obtained striking results with tomato and flax. Lipman and MacKinney (1931) also used crystallised salts, but the results were inferior to those of Sommer. No mention of the type of distilled water was given by Lipman and MacKinney, whereas Sommer used water from pyrex glass stills. Brandenburg (1933), (1935), and van Schreven (1936 a, b) obtained satisfactory results with copper deficiency in 3-4 weeks with cereals and beet by the use of recrystallised salts. The sparingly soluble calcium phosphate used by Brandenburg was prepared by precipitation from recrystallised calcium chloride and sodium phosphate. A similar method was used by Sommer and Sorokin (1928) and Piper (1941) (1942) in the preparation of calcium sulphate from calcium nitrate or chloride and pure or redistilled sulphuric acid. Arndt and Hoffman (1938) attempted to purify the iron and zinc compounds used in copper deficiency cultures

by recrystallisation. Piper (1942) (and private communication) found that twice-recrystallised salts were remarkably free from copper and often from other heavy metals. Saeger (1937) concluded that although copper was removed from calcium nitrate, magnesium sulphate and potassium tartrate by recrystallisation, this procedure was not effective with phosphates.

Hoagland *et al.* (1935), using an accepted micronutrient technique, observed responses to zinc deficiency in water cultures when using recrystallised salts, but effects were not severe for apricot until three months had elapsed. Roberg (1928) obtained improved results for zinc deficiency in *Aspergillus* by recrystallising the salts five times, but recrystallisation of the sugar (a troublesome source of impurities) was ineffective and an absorption technique was greatly superior for sugar purification. In a later paper Roberg (1932) did not consider that repeated recrystallisation was satisfactory for the removal of zinc or iron from the nutrients.

The removal of iron by recrystallisation from the macronutrient salts was not satisfactory according to Waring and Werkman (1942) and these workers regarded such methods as inefficient. Hopkins (1934) considered that recrystallisation was relatively ineffective for the removal of manganese as compared with an absorption technique. Piper (1941), however, found that twice-recrystallised salts were suitable for manganese deficiency in higher plants and a similar conclusion seems justified from unpublished work by the writer and others with cauliflower, oat and clover. Bertrand (1912) purified iron salts from manganese by recrystallisation of ferric ammonium sulphate. This salt was selected, as manganese cannot readily replace either the ferric or ammonium ion in isomorphous salts.

Little work has been published to indicate the value of recrystallisation for the elimination of molybdenum. Piper (1940) obtained a response to molybdenum with oat grown in nutrient solutions prepared from salts that had been crystallised during a more general procedure of purification by an adsorption process not specific for the removal of molybdenum. Mulder (1948 a) refers to his earlier experiments with *A. niger* where recrystallised salts had resulted in a greater growth depression than nutrients purified by a charcoal adsorption and considered that the difference was due to the *reintroduction* of molybdenum by the carbon. In later work (1948 a) he used selected salts based on their freedom from molybdenum as determined biologically. In the work of Hewitt and Jones (1947) (1949) (1950) referred to below and of Evans *et al.* (1950), the salts were first recrystallised before further treatment by more specific methods. Hewitt and Hallas (1951) found, however, that whereas recrystallisation effected some partial improvement in such compounds as magnesium sulphate or potassium nitrate, this was apparently not so for sodium phosphate (cf. Table 18). Possibly the formation of a phosphomolybdate complex prevents the separation of these elements when one is present in relatively small concentrations. Yields are, however, further reduced when specific purification methods are applied to recrystallised salts as compared with unrecrystallised salts, as shown in Table 18.

Piper (private communication) recommends recrystallisation of ferrous ammonium sulphate as a means to obtain iron compounds free from molybdenum.

Hutner *et al.* (1950) prepared pure potassium bicarbonate by precipitation from aqueous solution with the addition of propyl alcohol and examined whether preparation of calcite crystals could yield a pure calcium compound for cobalt deficiency.

Distillation Methods and Extraction of Solid Reagents

Distillation where feasible, as for example, for the purification of volatile acids and ammonia, appears to be a good method of purification and, subject to freedom from spray and creep, may be recommended for many separations according to Hughes (1943). Hutner *et al.* (1950) recommended the use of a vacuum-jacketed column packed with glass helix rings for preparation of pure nitric acid. Naturally, distillation is not applicable to separation from volatile impurities, for example boric acid or gallium salts. Calcium nitrate free of boron is, however, readily obtained by mixing reagent grade (already distilled) nitric acid and pure calcium carbonate, as shown by Hewitt (1946 a) (1947 a). A specialised grade prepared by ammonium carbonate precipitation and known as " fluorine free " is available* (cf. *Analyst*, 1944) and

* Messrs. B.D.H. Ltd., London.

is apparently almost boron-free as well as being probably free of a number of other impurities. Hutner *et al.* (1950) prepared purified phosphorus compounds by distillation of phosphorus oxychloride in silica. This was dissolved in water and the hydrochloric acid removed by distillation before addition of ammonia or potassium bicarbonate.

An application of distillation to the removal of volatile impurities lies in the distillation of the methyl borate ester. This was found to be readily volatile by Jaulmes and Galhac (1937) and might be adapted as an efficient means of purifying moderately concentrated solutions of sulphuric acid by repeated distillation after addition of methyl alcohol. Hewitt (unpublished) compared the effects of recrystallisation and a method based on the formation of the methyl borate ester for the removal of boron from solid nutrient salts. In the latter method the finely divided salts were refluxed several hours with absolute methyl alcohol saturated with dry hydrogen chloride and then the volatile fractions were distilled off. The procedure was tedious and not superior to recrystallisation. The treated salts tended to form a " rock " in the distillation vessel and magnesium sulphate *heptahydrate* was dehydrated to the *monohydrate*. Hughes (1943) has pointed out that attempts to treat solid compounds only dispersed in a liquid phase are not reliable and depend greatly on the fineness of division of the particles. The same criticism applies to continuous extraction of solids by liquids and this method cannot be recommended, although it was used by Steinberg (1936) (1938 a) for the attempted purification of sugars. Slight responses to zinc and molybdenum were observed and also to gallium, but this could not be confirmed in subsequent work, Steinberg (1945 a).

Carbon Adsorption Techniques

The adsorptive properties of carbon preparations have been employed by several workers for the removal of micronutrient impurities. The results have sometimes been conflicting and some uncertainty exists as to the appropriate application of these procedures. Other factors, such as pH of the solution, form of combination of the impurity to be removed and history of the carbon appear greatly to modify the results that may be obtained. A general study of the adsorptive properties of carbons has been made by King (1933) (1934 a, b) (1937) and Bennister and King (1938). The effects of temperature of activation, pH and porosity on the nature of materials adsorbed were studied. Carbons activated at high temperatures (*ca.* 700° C.) tend to adsorb acidic molecules and large molecules ; carbons activated at low temperatures (*ca.* 450° C.) adsorb smaller molecules and bases and have a smaller pore size. From pH 4·0 upwards activated carbons have a negative charge which increases with temperature of activation.

The use of carbons appears to have been adopted mainly by example from European investigators cited below in studies on the effects of zinc and copper on microorganisms. There appears to be no record of the use of carbons for the removal of boron. The influence of carbon on the removal of boron as a complex with manitol or other polyhydroxy derivatives would be worth investigation, since Vickery (1946) has recently found that rare earth separation by means of adsorption of their organic complexes on carbons is an efficient procedure. This field might be extended to other micronutrients as a matter of interest, although other existing methods are entirely suitable.

An early record of the use of carbon is that of Bortels (1927) in a study of the importance of iron, zinc and copper for microorganisms. The carbon method was claimed to be as effective as earlier methods of recrystallisation. A blood charcoal " *carbo medicinalis* " Merk, was employed. Neutral or alkaline solutions were necessary for efficient adsorption of iron and zinc. Removal of copper was less satisfactory, but was improved by the addition of ammonium sulphide.

Roberg (1928) used Bortels' (1927) method in a similar study of the iron, copper and zinc requirements of *Aspergillus*. *Carbo medicinalis* and *Carbo animalis* were compared for effectiveness and the former was markedly superior, especially for the removal of copper, and compared favourably in this respect with the phosphate adsorption method of Steinberg (below). In a later investigation with green algae Roberg (1932) was unable to produce copper

deficiency effects by use of Bortels' method with *Carbo medicinalis* at pH 8·0, although a further response was obtained for zinc and iron deficiencies, as compared with untreated nutrient solutions. Roberg (1931) examined more fully the action of carbons in the adsorption of micronutrients from culture solutions. The *Carbo medicinalis* was found to have 1·2-1·5% ash, mainly iron ; much of the iron was extractable by boiling in dilute hydrochloric acid The extracted carbon after washing with water was still active in adsorption of iron, zinc and copper, but adsorption was negligible at low or high pH values. In neutral solutions several carbons were found to be of equal efficiency for removal of zinc. Acidification only to pH 6·0 after carbon had been added caused relatively complete desorption of the adsorbed micronutrients and allowed normal growth. The amounts of carbon used in these experiments ranged from 2·5-5% of the weight of solution. Metz (1930) in similar experiments (for which no precise details were given) used 1-3% by weight of carbon. Lohman (1934) found that prolonged treatment for a period up to a maximum of 3-4 days with 5% of *Carbo medicinalis* at " a slightly alkaline reaction " was an efficient method for the removal of zinc in experiments with *A. niger*. Yields were reduced from 1,400 mg to 11 mg. *Carbo animalis* or *Carbo raffinis* were less efficient. Yoshimura (1934) found that all charcoals tested removed iron equally well, but that " medical " and blood charcoals were more efficient than sugar charcoal in the removal of copper. Gollmick (1935) also used *Carbo medicinalis* at 2·5% concentration at pH 7-7·5. The solution was allowed to remain in contact for several hours with frequent shaking and appreciable removal of zinc was obtained.

Lindenwood charcoal was used uncritically by Ondratschek (1941) after acid extraction and was found to contain less ash than other types. Yoshimura (1934) found that acid extraction of charcoals destroyed their ability to remove copper but not iron. Elevated temperatures also reduced efficiency.

Steinberg (1935) compared carbon treatment with various modifications of the phosphate adsorption method described below. No superiority was evident for the carbon method and it was frequently unreliable owing probably to the variable ash constituent of the carbon samples. Sakamura (1936) also compared results with carbon treatments obtained by other workers and by himself, with a calcium phosphate technique for the removal of iron, manganese, copper and zinc, in which the nutrients were boiled in the presence of 0·25-2·0% by weight of water-extracted calcium phosphate and then filtered. The carbon methods were all found to be inferior to the phosphate method. One carbon introduced additional manganese and another introduced zinc. Löhnis (1944) was also unable to remove manganese by " Norit " carbon ; use of the phosphate adsorption method was successful.

Mulder (1939 a, b) found " Norit," used in conjunction with a small quantity of " ammonium hydro-sulphide," was efficient for the removal of copper from nutrient media to be used for *A. niger*. The " Norit " added was only 0·5% by weight of the solution. Response to the addition of 0·1 μg Cu per litre could be detected. The same technique, however, was found by Mulder (1939 a) to introduce molybdenum into the solution. This was ascribed to the carbon but may have been due to the ammonium sulphide. Hewitt and Hallas (1951) has shown that use of this reagent as a source of sulphide ions prevented the production of molybdenum deficiency effects. Horner *et al.* (1942) found that filtration through carbon caused a partial removal of molybdenum and vanadium present in the sugars used for culture media to grow *Azotobacter*.

Phosphate, Alkaline Earth and Related Adsorption Methods

The application of alkaline earth and phosphate adsorption methods to the purification of plant nutrient solutions appears to date from the work of Steinberg (1919), although Bertrand and Javillier (1906) described a sensitive method for the precipitation of zinc ions by boiling with calcium carbonate or calcium salts and ammonia ; a compound with the composition $Ca\ (O\ Zn\ OH)_2 4H_2O$ was isolated. This method secured the precipitation of 90% of the zinc present at a concentration of 0·2 p.p.m. Steinberg's original method comprised autoclaving the complete nutrient solution (excluding any added micronutrients) with 15 g. calcium carbonate per litre for 20 minutes at 14 lb. pressure. The cooled solution was then

decanted from the solid material which contained the adsorbed micronutrients. This procedure was compared by Roberg (1928) with a carbon and ammonium sulphide treatment for removal of copper and found to be at least as efficient, but shaking alone with calcium carbonate without heating was ineffective. Steinberg (1935) found that steaming with magnesium carbonate could replace autoclaving with calcium carbonate. A higher pH was produced (*ca.* 9·0 instead of 7·5), but excess of magnesium carbonate removed all the phosphate from the medium. Use of magnesium carbonate was efficient in the absence of calcium ions. Filtration as soon as cool appeared to give improved results over long standing or the use of decantation. Comparison with the use of four different carbons did not indicate any striking differences, but the results with carbon purification were more variable ; some appeared superior and others inferior for removal of total metal impurities. No molybdenum was included in the medium but yields were normal (as judged from later work) when the remaining micronutrients were included irrespective of method of purification, except for the use of one carbon (decolorising charcoal) where only a 65% yield was produced.

Hopkins and Wann (1927) found that calcium phosphate ($Ca_3(PO_4)_2$) precipitated at pH 6 or over was an efficient means for the removal of iron from media used to grow *Chlorella*. Two solutions were prepared as follows :—

A. 5·9 g. $Ca(NO_3)_2$ 0·8 g. $MgSO_4$ 40 g. $C_6H_{12}O_6$ per litre.

B. 4·6 g. $K_2H\ PO_4$ per litre.

Equal volumes of A and B were mixed, shaken and allowed to stand for some days. The supernatant liquid was separated by siphoning. Hopkins (1934) used this procedure for the elimination of manganese from cultures of *Lemna*. The solutions were mixed hot and allowed to stand several weeks before filtration. Adsorption of manganese on ferric phosphate or zinc hydroxide was also effective, especially on the latter. Adsorption on added calcium phosphate (0·25-2·0% by weight of solution) at pH 5·5 was found by Sakamura (1936) to remove iron, manganese, zinc and copper consistently to less than 10^{-7} mol/litre. The calcium phosphate had first to be water-extracted and the pH was *raised* to 5·5 after addition of the phosphate, which was shaken with the solution for two hours and then filtered twice. The method was *ineffective* if the phosphate was added to an already alkaline solution which was subsequently acidified to pH 5·5. The calcium carbonate technique was also applied in similar investigations on *Phomatotrichum omnivorum* by Blank (1941) and for manganese deficiency studies by Löhnis (1944). The relative efficiency of the adsorption techniques discussed here is considered below together with their limitations.

Stout and Arnon (1939) and Piper (1942) developed independently similar modifications of the phosphate adsorption technique, which could be applied to the production of large batches of separately purified stock nutrients. Stout and Arnon (1939) inferred that maximum efficiency was due to the presence of an alkaline earth phosphate and an elevated pH obtained by the use of excess of an alkaline earth carbonate, and that the process consisted in the precipitation and adsorption of the metal impurities in the presence of phosphate *and* calcium ions. The procedure described by Stout and Arnon was as follows. Separate stock solutions of magnesium sulphate, potassium nitrate, calcium nitrate, diammonium phosphate or dipotassium phosphate were first prepared in 5-litre batches as 1- or 2-Molar solutions, according to solubility. To the phosphates was added 25 ml. of the 2-Molar calcium nitrate and 50 ml. was added to the magnesium sulphate. None was added to the potassium nitrate according to the original instructions (although this addition is recommended below) ; the calcium nitrate, of course, was already provided with calcium ions. To each of the stocks (except the phosphates) was added 50 ml. of Molar dipotassium phosphate and to all solutions was added 65 g. calcium carbonate. The mixtures were autoclaved at 20 lb. pressure for 1 hour, and filtered as soon as cool. Ammonium sulphate was treated as for magnesium sulphate, but steamed instead of autoclaved. The purified batches were tested by the dithizone test as a routine procedure and accepted if they contained less than ·01 p.p.m. of total heavy metals. After dilution into a nutrient solution this concentration was reduced to less than one-fiftieth of that value. Heavily contaminated batches were treated a second

time. Stout and Arnon (1939) found that a single treatment reduced the total heavy metal contamination from 0·5-5 p.p.m. down to ·01 p.p.m. or reduced the copper content from ·15 to ·001 p.p.m. or less.

The salts thus purified gave satisfactory and consistent results for manganese, copper and zinc deficiencies, which appeared in 3-4 weeks in tomato plants. The following data taken from Stout and Arnon (1939) indicate the efficiency of the method and, incidentally, the effect of distilled water.

Stout and Arnon (1939).

Treatment	Dry Wt. of whole plant g.			
	(1)	(2)	(3)	(4)
Complete nutrient ...	3·5	3·8	3·75	3·85
Copper deficiency ...	3·55	3·35	2·45	1·65
Zinc ,, ...	2·75	1·95	1·3	1·0
Manganese ,, ...	3·25	2·3	3·35	2·2

(1) Unpurified salts and ordinary distilled water.
(2) Purified salts and ordinary distilled water.
(3) Unpurified salts and glass distilled water.
(4) Purified salts and glass distilled water.

Piper (1942) precipitated the heavy metals by co-precipitation with tricalcium phosphate ($Ca_3(PO_4)2$) at pH 8·5, but specific details were not given; dipotassium phosphate presumably being used to produce the elevated pH. Two further stages were then included. These were an extraction with dithizone in carbon tetrachloride to remove the last traces of copper and zinc, and a final recrystallisation achieved by cooling to 0° C. the stock solutions already nearly saturated at room temperature. The crystals were dried in a cool oven. This technique was used for magnesium sulphate, potassium nitrate, sodium chloride, and dipotassium phosphate. Potassium dihydrogen phosphate was then prepared by acidification with redistilled nitric acid (and was thus an additional source of nitrogen to the extent of 1 mol per mol of phosphate). Calcium sulphate was prepared from calcium chloride purified as first described and from pure sulphuric acid.

Piper considered the production of the solid (crystallised salts) advantageous for two reasons, namely that less contamination was likely during prolonged storage and that a supply of solid salts comprised a reproducible stock for experiments carried out after prolonged intervals. Salts prepared thus were satisfactory for studies with copper and zinc deficiencies, and copper deficiency appeared in Algerian oat in 27 days (Piper (1942)). The dithizone extraction was not intended or claimed to have any influence on the manganese content of the solutions. Hageman *et al.* (1942), however, followed the use of the adsorption technique with an ineffective and unnecessary dithizone extraction to demonstrate only manganese deficiency in oat. Plants grown with the purified solutions contained 3 p.p.m. manganese, whereas those grown with unpurified salts contained 9 p.p.m.

Liebig *et al.* (1942) eliminated toxic concentrations of copper by the addition of calcium hydroxide solution to the nutrient in the presence of phosphate followed by removal of the supernatant liquid with a siphon.

The use of the adsorption method of Stout and Arnon (1939) is widespread and has now become the accepted routine procedure for the study of manganese, zinc and copper deficiencies in higher plants by many investigators, including Hoagland (1941), Millikan (1942) (1944), Wade (1944) and Hewitt (1945) (1946 a) (1947 a). Horner and Burk (1934) and Waring and Werkman (1942) found, however, that the iron requirements of bacteria were so low that the adsorption technique was inadequate for the removal of iron from the nutrients used for such work.

It is necessary to examine what other elements, including possible micronutrients, are removed by alkaline earth adsorption techniques. Steinberg (1938 b) claimed to have established the need for gallium by *A. niger*. The claim was based on the observation of somewhat low yields when nutrient cultures were prepared with spectrographically pure salts and

sugar extracted with alcohol. Yields were further reduced to 37·7% of the maximum when the adsorption technique was applied and only restored to the maximum by the addition of gallium. This claim was not confirmed by Steinberg (1945 a) in later experiments, after exhaustion of the particular batch of sugar, and has not been since confirmed in published work elsewhere. The essential nature of gallium therefore remains an open question. Liebig *et al.* (1943 b) carried out tests to determine the influence of gallium on *Citrus* and obtained negative results as far as deficiency effects were concerned. They concluded, however, from spectrographic tests that gallium is carried down by a phosphate precipitation. The culture solutions were purified accordingly by addition of calcium hydroxide to the phosphate-containing solution until precipitation occurred. The precipitate was analysed spectrographically. No gallium was detected and the nutrient accordingly contained probably less than 0·0005 p.p.m.

Some doubt has arisen as to the value of the adsorption technique just described for the removal of molybdenum. It is clear from the data presented by Steinberg (1936) (1937) (1945 b) some of which are given below in Table 16, that little benefit was actually obtained with respect to the removal of molybdenum by use of such procedures. Steinberg (1938 a) found no response to purification in experiments on the molybdenum needs of *Azotobacter* and *Rhizobium*. Arnon and Stout (1939 b), on the other hand, used their standard adsorption technique in experiments that showed the need of tomato for molybdenum and considered that the technique was necessary for " consistent results." The same technique was used by Hoagland (1941) to show the need of Myrobalan plum for molybdenum and also by Horner *et al.* (1942) for *Azotobacter*, but molybdenum still remained in the solution. Piper (1940) (1944) (and private communication), has pointed out that the adsorption technique is not specific and indeed is ineffective for the removal of molybdenum. Vanselow and Datta (1949) consistently failed to remove molybdenum by these methods. The same conclusion was reached from unpublished experiments by the writer where molybdenum was added to nutrient stock solutions, which were then treated by various modifications of the adsorption technique. Removal of molybdenum was not appreciable and never exceeded 20%. Further proof of the ineffectiveness of the method was obtained by Hewitt and Hallas (1951) by the use of *A. niger* (Table 18). The adsorption technique to remove molybdenum has now been superseded by workers of the California School and a sulphide co-precipitation method substituted, as stated below.

Hughes (1943) in his examination of purification procedures added a mixture of several heavy metals at 1 p.p.m. each to a 10% solution of pure potassium nitrate. Steinberg's technique using basic magnesium carbonate (already prepared pure) was applied, and the operations were repeated five times. Several metals, including aluminium, cobalt, chromium, manganese, nickel and vanadium, were removed completely at the first operation ; silver, copper and zinc required two repetitions for removal ; molybdenum and, incidentally, boron, were not removed at all after five repetitions.

It may be reasonably concluded that alkaline earth phosphate adsorption is an efficient means of removing simultaneously the greater part, but not all of the heavy metals that may be present, including the micronutrients iron, manganese, copper and zinc, but not molybdenum.

Slight modifications in procedure may cause variations in efficiency as shown by data of Steinberg (1938 b) (1945 b) in Table 16. This point was investigated further in unpublished work by the writer, and certain improvements were introduced as a result. The procedure finally recommended is given in detail in Part Two, but comprises the following essentials :— the presence of a soluble calcium salt, an alkaline phosphate, calcium or magnesium carbonate and sodium bicarbonate. Under these conditions the calcium bicarbonate present in solution before heating is decomposed to form what may be termed " nascent " calcium carbonate *in situ*, and increased adsorption efficiency is obtained. The data in Table 17 summarise the experimental results obtained with several trials. These included the following variables : presence of calcium ions or replacement by magnesium ions, presence of calcium carbonate or replacement by magnesium carbonate, presence of bicarbonate alone, or phosphate alone,

TABLE 16

Data of Steinberg on Effect of Nutrient Purification on Yields of A. niger *or* Lemna *after Omission of Various Micronutrients. (Figures as per cent. of yield in full culture)*

Steinberg (1936) *A. niger*				Steinberg (1937) *A. niger* Mo deficiency			Steinberg (1938 b) *A. niger*					Steinberg (1945 b) *Lemna*			
Omission	Unpurified	$MgSO_4$ replaced by $Mg(NO_3)_2$	Purified (adsorption method)	Source of N	Unpurified	Purified (adsorption method)	Omission	Unpurified	Boiled with CaO	Boiled with MgO	Autoclaved with CaO	Omission	Unpurified	Purified with $CaCO_3$	Purified with $CaHPO_4$
Fe	33%	17%	—	$Ca(NO_3)_2$ (a)	—	88·6%	Zn	1·9%	35·4%	4·6%	15·2%	Zn	74-91%	48·1%	63·0%
Zn	40%	7·8%	1·6-2·2%	,, (b)	—	93·2%	Cu	90·8%	74·5%	91·3%	59·3%	Cu	58-80%	35·8%	77·8%
Mo	95%	66%	91%	$NaNO_3$	8-16%	26·9%	Fe	45·3%	12·8%	71·1%	12·3%	Fe	6-20%	3·8%	7·4%
				KNO_3	—	14·6%	Mn	31·8%	32·5%	60·6%	22·0%	Mn	4-18%	6·3%	4·9%
				$Mg(NO_3)_2$	—	16·2%	Ga	79·6%	41·5%	79·1%	42·9%	Ga	92-98%	94·2%	80·9%
												Mo	22-66%	61%	90·7%

or both together. The respective reagents were alays added in the same order to standardise the effects of partial precipitation before autoclaving. This was done for 40 minutes at 125° C, and the solutions were filtered after standing overnight. The test solution was 20% potassium nitrate, to which a mixture of " impurities " was added comprising 8 p.p.m. iron, 4 p.p.m. manganese, 4 p.p.m. copper, 4 p.p.m. zinc. Semi-quantitative estimations of copper and zinc were made with dithizone and quantitative estimations of manganese were made with the tetrabase test described in Part Two.

The results for manganese removal are shown from the residual concentrations given in Table 17.

The following conclusions were reached :—

(1) Substitution of Mg^{++} for Ca^{++} is inefficient in the presence of $CaCO_3$ (with one exception) but reasonably efficient in the presence of $MgCO_3$.
(2) The presence of bicarbonate and phosphate together increases efficiency.
(3) There is no significant difference between use of $CaCO_3$ and $MgCO_3$, when Ca^{++} is present.

Best average results were obtained in the presence of phosphate, bicarbonate, Ca^{++} ions and $CaCO_3$, and corresponded to 99·6% removal of manganese.

TABLE 17

Residual Concentrations of Manganese as p.p.m. Remaining after Treatment by Various Modifications of the Phosphate Adsorption Technique.

		Residual Mn	Mean for
Group A.	Phosphate only		
(1)	$CaCO_3 + Ca^{++}$	0·081	
(2)	$CaCO_3 + Mg^{++}$	0·233	A
(3)	$MgCO_3 + Ca^{++}$	0·069	0·115
(4)	$MgCO_3 + Mg^{++}$	0·078	
Group B.	Phosphate and Bicarbonate		
(1)	$CaCO_3 + Ca^{++}$	0·024	
(2)	$CaCO_3 + Mg^{++}$	0·115	B
(3)	$MgCO_3 + Ca^{++}$	0·036	0·058
(4)	$MgCO_3 + Mg^{++}$	0·054	
Group C.	Bicarbonate only		
(1)	$CaCO_3 + Ca^{++}$	0·150	
(2)	$CaCO_3 + Mg^{++}$	0·088	C
(3)	$MgCO_3 + Ca^{++}$	0·070	0·095
(4)	$MgCO_3 + Mg^{++}$	0·050	

Mean for A, B and C together

(1)	(2)	(3)	(4)
0·085	0·152	0·059	0·061

Mean for A and B only, together

(1)	(2)	(3)	(4)
0·053	0·174	0·054	0·066

Significant difference (5%) between treatments ·053

General mean ·089

Co-precipitation with Organic Reagents or Sulphides

The application of organic chelating reagents or sulphides as co-precipitation reagents has received little attention in plant nutrition work until recently. The failure of the alkaline earth adsorption techniques led Hewitt and Jones (1947) (1949) to apply a new procedure for purification of nutrient reagents from molybdenum by a specifically effective method. This was based on the work of Scott and Mitchell (1943), who showed that almost quantitative recoveries at microgram concentrations of several microconstituents of plant ash could be obtained by co-precipitation with 8-hydroxyquinoline (oxine) and iron or aluminium. The elements thus concentrated efficiently included molybdenum, copper, zinc, cobalt, nickel, and

gallium. Later papers by Mitchell and Scott (1947) (1948) have extended the field to the use of mixed organic precipitants, including especially oxine, tannic acid, thionalid and cupferron. By these means quantitative recoveries of gallium, and vanadium as well as of others were obtained. The methods seemed particularly useful, as they were especially designed to separate the microconstituents from compounds of potassium, sodium, calcium, magnesium and phosphorus, and were applied also for the removal of zinc by Nicholas and Fielding (1951).

The method used by Hewitt and Jones (1947) (1949) and followed also by Evans *et al.* (1950) consisted in the preparation of molar or 2-molar stock solutions of the principal nutrients which were acidified to pH 1·8 followed by addition of oxine (about 3-500 mg) and ferric chloride equivalent to 95% of the oxine added. The pH was raised to 5-5·5 by electrometric titration with analytical reagent grade 1·5 N. potassium hydroxide. The precipitate was left to stand overnight and filtered off. A chloroform extraction removed the last traces of oxine ; the residual chloroform was removed by warming the solution on a hot plate after extraction with ether. This method was tested by the *A. niger* technique by Hewitt and Hallas (1951) and it was found that removal of the greater part of the molybdenum impurity had been achieved, as shown by the data in Table 18. The relative efficiency of the numerous variations of the technique developed by Mitchell and Scott (1947) (1948) still remains to be tested by this sensitive procedure.

The development of specific methods for the removal of molybdenum was carried further by Stout and Meagher (1948) who mentioned the use of copper sulphide as a co-precipitant. Co-precipitation by copper sulphide has now superseded the use of oxine at Long Ashton in the work of Hewitt and Jones (1950) (1952). This technique is highly efficient for the elimination of molybdenum, is simple to carry out, is free from toxic effects if handled properly, and can be applied to the treatment of all macronutrient salts including phosphates, and also of manganese salts. The procedure worked out by the writer for use at Long Ashton is given in Part Two, and consists essentially in the precipitation of copper present at an initial concentration of 500 p.p.m. by hydrogen sulphide filtered through cotton wool to reduce spray. The precipitate is filtered off and the filtrate is warmed on a hot plate to remove residual hydrogen sulphide and to complete the precipitation of colloidal sulphides and, if necessary, is refiltered. The efficiency of the method may exceed 99·9% as shown in Table 18. The data in Table 18 show that the copper sulphide method is slightly more efficient than the oxine method. In a recent note Nicholas and Fielding (1950) also recommend the use of co-precipitation with mixed copper and lead sulphides at 250 p.p.m. initial copper and lead concentrations respectively. Nicholas and Fielding (1950) also state that 10 μg. molybdenum is added to 200 ml. of nutrient medium prior to purification, presumably to act as a self-collecting agent. Data presented below, however, clearly show that this procedure is open to criticism as the removal of molybdenum is probably dependent on an adsorption isotherm and in any case the residual concentration is a positive function of the initial concentration. Data of Hewitt and Hallas (1951) :—

Mo added per 50 ml. of Medium before purification	Mean Yield of Mycelium	Calculated Residual Mo Concentration	% Removal
0·25 μg.	71 mg.	0·000045 μg.	99·98
1·0 ,,	162 ,,	0·00051 ,,	99·95
4·0 ,,	400 ,,	0·0020 ,,	99·50

These results apply to co-precipitation of molybdenum in the presence of 500 p.p.m. copper. Comparable results at different levels of efficiency were obtained at 25, 100 and 1,000 p.p.m. copper where it was shown that increased copper concentration resulted in correspondingly increased efficiency of removal.

Co-precipitation of molybdenum by other sulphides, including those of lead, cadmium, mercury, silver, arsenic and tin, has also been examined by Hewitt and Hallas (1951). The relative efficiency of these co-precipitants was assessed by growth tests with *A. niger* grown in molybdenum-deficient cultures. Data in Table 18 indicate that copper sulphide was probably at least as efficient as the others and more so than some, for example cadmium sulphide alone, which was also toxic. A further variation was the use of ammonium sulphide

solution instead of hydrogen sulphide. This was found to be relatively ineffective as shown by the results in Table 18. Mixed sulphides of copper and lead or copper and cadmium were also efficient co-precipitants.

Sandell (1944, pp. 18-25) gave a useful summary of the principles of co-precipitation phenomena as applied to quantitative analytical methods, and there is evidently scope for further study of the application of these methods to the purification of nutrient reagents. Feigl (1949, pp. 163-164, 467-468) gave further details with special reference to co-precipitation by sulphides. Work reviewed showed that sulphide co-precipitation is somewhat specific. Copper is a good collector for molybdenum, lead and zinc, whilst mercury sulphide collects lead but is less effective for silver and copper. The behaviour of cadmium recorded here may thus be in harmony with the data given by Feigl. The precipitation of molybdenum as sulphide is greatly enhanced according to Feigl (1949) *loc. cit.*, by inclusion of a small amount of iodine. Under these circumstances hydrogen sulphide forms colloidal sulphur and the molybdenum sulphide is coagulated completely and readily filtered. Hydrogen sulphide alone under pressure may produce a similar response. The iodide might require removal prior to use. Hewitt and Hallas (1951) failed to detect any advantage from the inclusion of iodine, which was recommended by Nicholas and Fielding (1951) except for glucose, where colloidal suspension is prevented. Nicholas and Fielding (1951) suggested co-precipitation by cadmium sulphide for the removal of copper. Combined use of oxine and sulphide co-precipitation was used by Hewitt and Bolle-Jones (unpublished) for attempted removal of cobalt, nickel and gallium.

Extraction with Immiscible Solvents

(a) *After chelation.* The plant micronutrients and related elements of biological interest form chelate compounds with several organic reagents and many of these compounds can be readily extracted in immiscible organic solvents owing to the favourable partition co-efficients. This field has wide possibilities and much valuable information on the properties of these reagents and the separations obtainable by their use can be found in books by Sandell (1944) and Welcher (1947), where extensive and detailed treatments are devoted to oxine, cupferron, dithizone and other important reagents. Information on the application of chelation and extraction with oxine to the separation of iron, molybdenum and gallium is also given in papers by Moeller (1943 a, b) and Nierecker and Treadwell (1946). Albert and Gledhill (1947) and Albert and Magrath (1947) made a special study of oxine derivatives and other chelating reagents as related to metals of biological importance, and their observations might be applied to the special application discussed here. The work of Albert *et al.* was, however, directed to the application of chelating reagents as metabolic inhibitors and this aspect of the use of such reagents in purification procedure must be borne in mind. Any excess of chelating reagent left after purification may interfere with delicate tests with microorganisms, as was shown by the work of Zentmyer (1944) and may also, if residual traces of heavy metals are merely rendered unavailable, give a false impression of the efficiency of a purification technique based on the use of such reagents. Heck and Bailey (1950) found, however, that low concentrations of chelating agents added to culture solutions did not satisfactorily " block " the activity of micronutrients and higher concentrations exerted a directly toxic action on roots.

Illustrations of the use of extraction procedures for the removal of micronutrients are numerous. Arndt and Hoffman (1938) used extraction by dithizone in carbon tetrachloride to purify basal nutrient salts from copper, and Smith and Bayliss (1942) used the same reagent in chloroform for the removal of zinc. Smith and Bayliss emphasised that the use of purified dithizone is particularly important as this reagent may be heavily contaminated with heavy metals, of which some were not readily removed by an alkaline extraction. Piper (1942) as already mentioned, used repeated dithizone extractions for removal of heavy metals after preliminary treatment of the nutrients with the phosphate adsorption method, for an investigation of copper deficiency in higher plants. Nicholas and Fielding (1948) (1951) found the same method necessary for production of copper and zinc deficiencies with *A. niger*. Hughes (1943) confirmed the effectiveness of dithizone in chloroform as a means of removing traces of copper and zinc from compounds of spectroscopic purity.

TABLE 18

Examples of Molybdenum Content in Culture Materials as Determined by Microbiological Assay with A. niger

(*Data of Hewitt and Hallas*, 1951, *and unpublished*)

Material tested	Weight of sample g.	Approximate dry weight Yield of mycelium mg.	g Mo assayed (determined as stated below*)	p.p.m. Mo
I. *Sand.* (Available Mo)				
a Bedfordshire Sand Double Arches Pit (Main) (Leighton Buzzard)	†{2 ·25	†380–760 100	·0014–·0042 ·00018	†·0007–·0021 ·0007
Double Arches Pit (Front)	2	290	·001	·0005
b Loch Aline sand	†2	†320–780	·0012–·0043	†·0006–·0022
c Norwegian quartz	2	600–700	·003–·0036	·0015–·0018
d Norfolk sand (Leziate nos. 851, 852, 853)	5	400–850	·0016–·0055	·0003–·0011
Removal of molybdenum during purification of Bedfordshire sand				
(i) after first treatment with acid	2	160	·0004	·0002
(ii) ,, second ,, ,, ,,	2	80	·0001	·00005
(iii) ,, third ,, ,, ,,	2	60	·00004	·00002
(iv) sand treated 4 times (3 acid + 1 alkali)	†10	†16–42 (6 replicates)	0–·00002	†·00001 (Mean)
II. *Water.*				
a Water redistilled once from pyrex glass stills				
(i) from carboy retained for — Mo work	40	80	·00012	·000003
(ii) from carboy used for + Mo work	40	160	·0004	·00001
b Water distilled from metal stills with silver condensers (as used at Rothamsted)	40	500	·0025	·00006
c Rain water (stored 12 months)	10	460	·0018	·00018
d Rain water demineralised by treatment with one anion-exchange unit. De-Acidite E.§	30–40	350–480	·0013–·0022	·00005 = 72% removal
e Tap water	10–40	350–850	·001–·005	·00013
f Water containing ·04 μg. Mo per 5 ml. demineralised with Bio-Deminrolit§ or IMAC-c-11§	5 5	26–46 30–38	<·00003 <·00003	<·000005 <·000005 =>99·9% removal
III. *Nutrient reagents and effect of recrystallisation*				
a Analytical reagent grade KNO_3	·25	— 190	— ·0008	— ·003
b ,, ,, ,, $MgSO_4$	·05	— 890	— ·006	— ·15
c Recrystallised grade NaH_2PO_4	·125	— 160	— ·0006	— ·005
d A.R. KNO_3 twice recrystallised	·25	†330 60	·00125 ·00015	†·005 ·0006
e A.R. $MgSO_4$ thrice recrystallised	·05	†140 210	·00034 ·0009	†·009 ·026
f A.R. NaH_2PO_4 twice recrystallised	·125	†730 412	·0038 ·002	†·03 ·016
IV. *Effects of purification*				Apparent efficiency as % removal of Mo
a Complete nutrient solution prepared with salts *a*, *b*, *c*. Recrystallised only		990	·01 or greater	
b Complete nutrient solution purified with copper sulphide precipitated by ammonium sulphide		550	·0027	70 approx.
c (i) Complete nutrient solution purified with copper sulphide precipitated by H_2S.		†20–55 (usually 35)	<·00005	>99·5 ‡99·9 or over actually determined
(ii) As above but unrecrystallised salts used		90	·00025	—
d As above using cadmium sulphide		730	·004	60
e As above using lead sulphide		230	·0008	92
f As above using lead plus cadmium sulphides		56	<·00005	>99·5
g As above using iron and 8 hydroxyquinoline		160	·0004	96
h As above using the phosphate adsorption method		700	·004	60 approx.

† Independent determinations on separate samples.

* The mean Mo value was determined from the individual values corresponding to three replicate cultures and not from the mean of the dry weights, and referred to calibration growth data obtained each time.

‡ The values of 99·91–99·98% removal were determined in a series of tests in which known amounts of molybdenum were added to purified solutions which were then repurified.

§ De-Acidite E and Bio-Deminrolit were supplied by the Permutit Co., Chiswick, London.

IMAC–c–11 was supplied by Messrs. Industrieele Activit, N.V. Amsterdam. The results shown were obtained at pH 2·0. Efficiency of removal was slightly reduced at higher pH values (4–8).

[*To face page* 80

Extraction with oxine in chloroform was first used by Waring and Werkman (1942) to remove iron from culture media used for bacteria. These workers found that the phosphate adsorption technique, and precipitation or recrystallisation methods were inadequate for removal of iron in their experiments owing to the extremely low level required by bacteria, which was estimated at only one-tenth that needed by *A. niger*.

Gale (1949) used the same technique to prepare bacterial media free from manganese, magnesium and cobalt. The extractions were repeated five times at an alkaline pH. Nicholas and Fielding (1948) prepared nutrient reagents relatively free from molybdenum by extraction with oxine and chloroform. A final extraction with ether is often helpful as this removes the remainder of the chloroform more readily than warming, which, however, quickly disposes of the residual ether.

Cupferron in chloroform was tested by Hewitt (unpublished) as a possible method for the removal of molybdenum, with inconclusive results. Bertrand (1941) used the same reagent at pH 1·8 to purify nutrient media from vanadium and claimed that this technique showed the need of *A. niger* for this element. The solutions purified in this way were stated to contain less than 1 μg. per litre of vanadium.

(b) *Extraction with ether-hydrochloric acid.* A useful separation of iron from manganese, copper, zinc and several other elements can be achieved by extraction of concentrated solutions of ferric iron salts with ether in the presence of hydrochloric acid. The solubility of the ferric chloride in ether increases as the hydrochloric acid increases in concentration up to 6N, after which there is little change. This method was originally devised by Rothe and is discussed at length by Fales (1928, p. 273), Hillebrand and Lundell (1929, p. 106) and by Sandell (1944). The method was used by Piper (1941) (1942) (and private communication) to prepare a source of ferric citrate free from manganese, copper and zinc and was also recommended by Mitchell and Scott (1947) (1948) for preparation of spectroscopically pure iron compounds. Ferric ammonium sulphate is the most satisfactory starting point and the procedure as suggested by Piper is given in Part Two. The use of ether-extracted ferric chloride as a source of iron compound does not appear to have been widely used, and in fact, the use of purified iron compounds is still relatively uncommon.

The ether-hydrochloric acid method unfortunately also concentrates molybdenum and gallium in the ether phase according to Fales (1928), Hillebrand and Lundell (1929) and Sandell (1944), and is unsuitable in that form for the more complete purification of iron compounds. Hewitt and Jones (1949) (1950) (1952) have described a modified method devised by the writer whereby gallium and molybdenum are also removed. This is possible because ferrous iron is not extracted into the ether phase according to Sandell (1944) (1947), and molybdenum and gallium are removed. The ferrous iron is then oxidised to the ferric state by pure (vacuum distilled) hydrogen peroxide prior to a second ether extraction, which then separates iron from the other micronutrients. The full method is given in Part Two. The ether extraction method might also be applied in instances where it was desired to separate tungsten and vanadium from molybdenum or iron. Lingane and Meites (1946) found that tetravalent vanadium is retained in the aqueous phase when peroxide-free ether is used. Toluene 3 : 4 : dithiol (Dithiol) reacts with molybdenum, and this reagent was used for the purification of iron compounds from molybdenum by Hewitt and Jones (1947). This reagent in amyl acetate extracts molybdenum from solutions of iron compounds in the presence of hydrochloric acid of S.G. 1·075 or over, the experimental details being determined by Wells and Pemberton (1947). It may be expected that the application of other extraction methods will prove of value in the further purification of basal and micronutrient salts used to test the responses of plants to other, as yet unproved, micronutrients.

Other Methods of Purification

A few isolated methods not easily classified merit attention here. Bertrand (1912) prepared basal nutrient salts free from manganese by addition of ammonium hydroxide followed by oxygenation ; the colloidal $MnO_2.H_2O$ was then carried down in the presence of magnesium ammonium phosphate, which was precipitated when equivalent amounts of magnesium

sulphate and ammonium phosphate were added to the solution. In experiments on the zinc requirements of *A. niger* Bertrand and Javillier (1911) purified manganese sulphate from zinc by reduction of potassium permanganate with sulphur dioxide to the manganese dioxide stage. This was collected by filtration and redissolved in sulphuric acid. Precipitation of manganese hydroxide by ammonia was rejected, as zinc is co-precipitated. Wolff and Emmerie (1930) attempted to purify basal nutrients by electrolysis with platinum electrodes in the presence of dilute sulphuric acid, but the results appeared relatively unsatisfactory as judged by the response of *A. niger*.

Samuel and Piper (1929) prepared ferric citrate free from manganese by electrolytic deposition of iron from ferrous sulphate on to a platinum electrode. The iron was dissolved in hydrochloric acid and then converted to citrate. Piper (1941) in a later investigation used ferric citrate prepared from ferric chloride that had been ether-extracted as described above, but prior to this the chloride had been obtained from ferrous sulphate with the intermediate precipitation of the iron by hydrogen sulphide. Saeger (1937) also used hydrogen sulphide precipitation to remove copper after a preliminary precipitation of ferric chloride by ammonia and Chapman *et al.* (1937) used hydrogen sulphide to eliminate zinc from ferrous sulphate. Hewitt and Jones (1947) removed molybdenum from ferric citrate first by extraction with toluene 2 : 4 : dithiol in amyl acetate and hydrochloric acid, followed by co-precipitation of molybdenum with a small quantity of oxine. The bulk of the iron not precipitated thus was precipitated with ammonia and the washed precipitate was dissolved in citric acid. Evans *et al.* (1950) used a thiocyanate-ether extraction in the presence of sodium sulphide to remove molybdenum from ferrous sulphate. The iron was then precipitated with ammonia and redissolved in N. HCl.

Hageman *et al.* (1942) prepared iron salts free from manganese by the method of Ray (1940). This involves the precipitation of ferric iron by addition of pyridine to ferric nitrate, and is said to avoid the co-precipitation of manganese. Hutner *et al.* (1950) pointed out that volatilisation of iron carbonyl or ferric chloride was sometimes effective. Archibald (1932) outlined a procedure for the preparation of iron free from several heavy metals. The first stage was a sulphide precipitation which removed copper, lead, tin and bismuth. This was followed by precipitation of ferric hydroxide at an alkaline pH to separate iron from zinc, cobalt and nickel. The ferric hydroxide was redissolved and electrolysed as the oxalate to eliminate manganese in a manner analogous to that of Samuel and Piper (1929).

Raleigh (1939), in a study of what he regarded as silicon-deficiency in beet, purified potassium silicate by reaction with hydrofluoric acid followed by hydrolysis of the silicon fluoride in pure ammonia solution. The silica was filtered off and fused with " pure " potassium carbonate. No molybdenum was included in the nutrient solution and the basal salts were untested C.P. grade. Vanselow and Datta (1949) attempted to prepare calcium and magnesium nitrate free from molybdenum by dissolving freshly precipitated and washed hydroxides in redistilled nitric acid, with inconsistent results.

An interesting method of purification of the non-ionic fraction of a bacterial medium is due to Perlman (1945) who used synthetic resin ion exchange columns to remove cations. Hewitt (1950 unpublished) found that synthetic resin ion-exchange materials were able to remove 70-99·9% of added molybdenum when glucose was perfused through columns of these materials at pH 2·0. Ion-exchange columns were also tested by Nicholas and Fielding (1951) for similar reasons, but chemical methods were preferred.

Summary of Recommended Methods

The survey concluded above suggests that certain methods are more effective than others for the removal of particular micronutrient elements and the following conclusions are suggested :—

(1) Recrystallisation is probably the most appropriate method for removal of boron and is also moderately efficient for copper and manganese.

(2) Carbon adsorption methods seem to be somewhat unreliable, but may be effective for the removal of copper.

(3) Alkaline earth phosphate-carbonate adsorption techniques are a good general method for reduction in impurities of iron, manganese, copper and zinc, and also cobalt.

(4) Sulphides and 8 hydroxyquinoline (oxine) are efficient co-precipitants for molybdenum, and oxine and other organic reagents may be especially useful where gallium and many other microelements are concerned.

(5) Extraction with dithizone is a good means of eliminating copper and zinc, especially in conjunction with alkaline earth adsorption techniques.

(6) Extraction with " oxine," cupferron or dithiol merits attention for the removal of iron, molybdenum and gallium and possibly other elements.

(7) Extraction with 6N hydrochloric acid and ether is a satisfactory method for the separation of iron from manganese, copper and zinc, and can be adapted for the elimination of molybdenum and gallium.

Selected applications of these methods are detailed in Part Two.

THE COMPOSITION OF THE NUTRIENT SOLUTION

Since almost as many different nutrient solutions have been devised as there have been experiments it would be pointless to attempt to illustrate more than a small proportion. This section is intended therefore mainly to illustrate, with examples, the more important variations in nutrient composition and to consider the effects of source of nitrogen, salt concentration, pH, supply of iron and incidence of iron deficiency, and the supply of micronutrient elements and of other supplements. The application of nutrients is discussed later.

Terminology

The specification of nutrient concentration has been given in various ways and these include the use of grams per litre (frequently in round figures as in Crone's solution and many others) ; parts per million (again frequently in round figures) ; concentration ratios, e.g. 1/10,000 $CuSO_4 5H_2O$; molar fractions ; milligram equivalents per litre, and gram ion fractions. Since there can be only one weight of an element corresponding to any given concentration, whatever terminology is selected, any of them is equally accurate and they are all interconvertible. Some descriptions are, however, more fundamental and useful than others. The use of concentration ratios as, for example, was adopted by Brenchley (1910) (1914) and Warington (1923) (1937) to describe limits of toxicity or deficiency of micronutrient metals is, in the opinion of the writer, neither convenient nor fundamental. Specification of concentration as 1/10,000 $CuSO_4 5H_2O$, i.e. 0·1 g/L., does not readily convey to the mind the actual concentration of the *metal* whose activity is under consideration and the units employed bear no relation to the molecular or equivalent weight of the compound used. The same criticism applies to the use of the g/L terminology, e.g. 0·5 g/L $CaSO_4 2H_2O$ for macronutrient salts, except where the weight is a *specified* molar fraction, e.g. 0·246 g/L $MgSO_4 7H_2O$, which represents 0·001 mol. fraction or 1 millimol. The use of the parts per million terminology has only the advantage of conveying to the mind the exact concentration of the separate elements in terms of concentrations of physiological value, and enables those experienced in the study of plant nutrition to appreciate at a glance the significance of a concentration stated thus. For this purpose it is still generally used to specify levels of the micronutrients, where these do not comprise an important variable. Use of the parts per million terminology has been criticised by Livingstone and Tottingham (1918) on the grounds that it is unscientific, non-fundamental and confusing. The use of g. molar (referred to often as partial volume molecular concentrations) and g. ion fractions has much to commend it, and was advocated or adopted by Knop (1861), Nobbe (1865), Tottingham (1914), Shive (1915 a, b) *et seq.* McCall (1916 a), Livingstone (1942) and others. This terminology is based on fundamental concepts and encourages thinking in terms of the true chemical composition of the solution. The preparation of nutrients is greatly facilitated by taking suitable aliquots or multiples of molar or 2-molar stock solutions and calculation of awkward fractions of parts per million are avoided with consequent saving of time and effort. This terminology has in fact even been

applied to the specification of very dilute concentrations of potassium as low as ·000014 M.K_2SO_4 (1·1 p.p.m. K) by Ahlgren (1941) and ·00004 M. K_2SO_4 (3·2 p.p.m. K) by Wall (1940). An alternative to the use of molar fractions is the milligram equivalent per litre terminology. This was recommended by Hoagland and Martin (1923) and is extensively used. It has the advantages of the preceding one in retaining the fundamental approach and of taking into account the valency of the respective ions including those where change of valency occurs, e.g. iron. In these instances complete specification must include both the equivalent concentration (1 m. eq./L. = 10^{-3} g. eq./L.) and the valency. Use of this terminology is more logical when such problems as the iron-manganese ratio are considered since expression of the ratio as Fe^{++}/Mn^{++} or Fe^{+++}/Mn^{++} in terms of equivalents gives a clearer presentation of the state of affairs than a ratio of, say, 2 : 1 as parts per million of Fe and Mn. Both were used by Chandler (1941) to specify micronutrient concentrations. For the reasons given above the guiding terminology adopted here is that of the milligram equivalent per litre (m.eq./L) with statement also of the concentration as parts per million as a convenient alternative. For reasons of almost universal usage the use of parts per million has been retained for the micronutrient supplements, except where these constitute a principal variable, e.g. in experiments on Fe/Mn or Mo/Mn ratios.

A typical modern nutrient solution might be specified therefore as follows :—

Molar :—KNO_3·006, $Ca(NO_3)_2$·004, $(NH_4)H_2PO_4$·001, $MgSO_4$·002 ;

p.p.m. :—Fe 5·6, Mn ·55, Cu ·064, Zn ·065, B ·37, Mo ·019,
or

m.eq./L :—K^+6, Ca^{++}8, Mg^{++}4, NH_4^+1, (H^+2), NO_3'14, PO_4'''3, SO_4''4;
Fe^{+++} ·3, Mn^{++} ·02, Cu^{++} ·002, Zn^{++} ·002, BO_3''' ·1, MoO_4'' ·0004 (Mo^{VI} ·0012)

A recent suggestion by Schuffelen and Lossjes (1942) advocates the use of activities rather than molar concentration. This has the merit of being probably the most fundamental approach to the representation of the properties of a nutrient solution as they affect absorption by the plant. The concept, however, may require greater familiarity with physical chemistry than is convenient and difficulties in determining reliably the activities of the chemical ions in a complex mixture should not be underestimated.

Examples

The data in Tables 19 A & B include a wide variety of nutrient solution types. For the sake of uniformity and comparison the data for the macronutrients have been converted into parts per million and milligram equivalents per litre where necessary from the respective authors' original figures. The original reference to each is also given. Some of the solutions shown are now of historical interest, others have unusual features such as the use of sparingly soluble salts, and some represent well tried and highly satisfactory solutions, examples of which are purposely included in the historical section of Table 19 A to illustrate the evolution of the modern type of nutrient. A limited number of selected nutrient solutions are also given below to show the actual composition in g/L of the respective nutrient salts as originally published. The composition of the Long Ashton Solution is given in Part Two.

Composition of some Nutrient Solutions illustrating the Compounds used

Knop's Solution (1865)	g/L	*Crone's Solution* (1904)	g/L
KNO_3	0·2	KNO_3	1·0
$Ca(NO_3)_2$	0·8	$Ca_3(PO_4)_2$	0·25
KH_2PO_4	0·2	$CaSO_4$	0·25
$MgSO_4 7H_2O$	0·2	$Fe_3(PO_4)_2 7H_2O$	0·25
$FePO_4$	0·1	$MgSO_4 7H_2O$	0·25

Wallace (1924)

	g/L
KNO_3	0·2
$NaNO_3$	0·5
K_2HPO_4	0·1
$CaSO_4 2H_2O$	0·1
$MgSO_4 7H_2O$	0·1
NaCl	0·1
$FeCl_3$ (anhyd.)	0·04

Wallace (1930)

	g/L
KNO_3	0·8
KCl	·038
K_2HPO_4	·099
$CaSO_4 2H_2O$	0·258
$MgSO_4 7H_2O$	0·3
$FeCl_3$ (anhyd.)	0·04

Hoagland and Snyder (1933)

	g/L	Molar
KNO_3	0·51	·005
$Ca(NO_3)_2$	0·82	·005
$MgSO_4 7H_2O$	0·49	·002
KH_2PO_4	0·136	·001

Ferric tartrate 1 ml/L of 0·5% solution.

Micronutrients A—Z solution shown below

Trelease and Trelease (1933)

	g/L	Molar
KNO_3	0·683	·0067
$(NH_4)_2SO_4$	0·0679	·00059
KH_2PO_4	0·3468	·00255
K_2HPO_4	0·01253	·000079
$CaCl_2$	0·4373	·00394
$MgSO_4 7H_2O$	0·7478	·00263
$FeSO_4 7H_2O$	0·00278	·00001
pH	5·1	

The Rothamsted Solutions. (*Courtesy of Dr. W. E. Brenchley*)

g/L.	*a*	*b*	*c*	*Modified Crone's*
KNO_3	1·0	1·0	1·0	1·0
$MgSO_4 7H_2O$	0·5	0·5	0·5	0·5
KH_2PO_4	0·45	0·4	0·3	—
K_2HPO_4	0·0675	0·135	0·27	—
$CaSO_4 2H_2O$	0·5	0·5	0·5	0·5
$Ca_3(PO_4)_2$	—	—	—	0·25
$Fe_3(PO_4)_2 7H_2O$	—	—	—	0·25
$FeCl_3$	0·04	0·04	0·04	—
H_3BO_3	0·001	0·001	0·001	0·001
$MnSO_4 4H_2O$	0·001	0·001	0·001	0·001
pH approx.	4·5	5·5	6·2	

Arnon (1938)

	g/L	Molar
KNO_3	0·612	·006
$Ca(NO_3)_2$	1·312	·008
$NH_4H_2PO_4$	0·115	·001
$MgSO_4$ (as $MgSO_4 7H_2O$)	0·49	·002

$FeSO_4$	0·5%	0·6 ml/L 3× weekly
Tartaric acid	0·4%	
H_3BO_3		2·86 mg.
$MnCl_2 4H_2O$		1·81 mg.
$CuSO_4 5H_2O$		0·08 mg.
$ZnSO_4 7H_2O$		0·22 mg.
H_2MoO4H_2O (85% MoO_3)		0·09 mg.

Arnon and Hoagland (1940)

	g/L	Molar
KNO_3	1·02	·01
$Ca(NO_3)_2$	0·492	·003
$NH_4H_2PO_4$	0·230	·002
$MgSO_4$ (as $MgSO_4 7H_2O$)	0·49	·002

Iron and micronutrients as for Arnon (1938) or as described.

The composition of the $A_4(A_5)$ B_7 and C_{13} microelement supplements is given overleaf.

Shive and Robbins (1942) *I.*

	g/L	Molar
$Ca(NO_3)_2$	0·938	·0045
$(NH_4)_2SO_4$	0·0924	·0007
KH_2PO_4	0·313	·0023
$MgSO_4 7H_2O$	0·567	·0023
$FeSO_4 7H_2O$	5·5 mg.	
H_3BO_3...	0·57 mg.	
$MnSO_4 4H_2O$	0·57 mg.	
$ZnSO_4 7H_2O$	0·57 mg.	

Shive and Robbins (1942) *II.*

	g/L	Molar
$NaNO_3$	0·34	·004
$CaCl_2$	0·1665	·0015
KH_2PO_4	0·214	·0015
$MgSO_4 7H_2O$	0·514	·0022
Iron and micronutrients as in I.		

Piper (1942)

	g/L
KNO_3	1·0
KH_2PO_4	0·5
NaCl	0·1
$CaSO_4 2H_2O$	0·5
$MgSO_4 7H_2O$...	0·5
Ferric citrate	0·02
H_3BO_3	0·5 mg.
Mn (as $MnSO_4$)	0·5 mg.
Zn (as $ZnSO_4$)	0·2 mg.
Mo (as Na_2MoO_4)	0·1 mg.
Cu (as $CuSO_4$)	0·003 mg. upwards

Robbins (1946)

	g/L	Molar
KNO_3	0·408	·004
$Ca(NO_3)_2$	0·820	·005
KH_2PO_4	0·136	·001
$MgSO_4 7H_2O$	0·493	·002
Fe	0·5 mg.	
B	0·25 mg.	
Mn	0·25 mg.	
Zn	0·25 mg.	
Cu	0·02 mg.	
Mo	0·01 mg.	

Sideris and Young (1949) (Suitable for growth of pineapple in water cultures)

NO_3 Type

	g/L	Molar
$Ca(NO_3)_2 4H_2O$	0·236	·001
KH_2PO_4	0·068	·0005
K_2SO_4	0·174	·001
$MgSO_4 7H_2O$	0·246	·001
$Na_2B_4O_7 10H_2O$	0·0038	·00001
$ZnSO_4 7H_2O$	0·0028	·00001
Iron ·5–5·0 μg and Mn 5 μg		

NH_4 Type

	g/L	Molar
$(NH_4)_2SO_4$	0·132	·001
KH_2PO_4	0·0685	·0005
K_2SO_4	0·174	·001
$CaSO_4 2H_2O$	0·172	·001
$MgSO_4 7H_2O$	0·246	·001
$Na_2B_4O_7 10H_2O$	0·0038	·00001
$ZnSO_4 7H_2O$	0·0028	·00001

The wide range of concentrations of the several nutrient elements comprised in a nutrient solution is obvious from Table 19. The normal concentrations of the principal macronutrients range from 2-8 m. eq. K^+, 6-10 m. eq. Ca^{++}, 1-4 m. eq. Mg^{++}, 5-15 m. eq. NO_3', 0-3 m. eq. NH_4^+, 2-4 m. eq. PO_4''', 1-4 m. eq. SO_4'' per litre. These may vary according to age or species and their uptake may be accomplished mainly during early growth in some instances, e.g. phosphorus, Gericke (1925 b), Brenchley (1929). Generally the level of Ca^{++} should not fall below 4 m. eq./L., although there may be exceptions (Warington, 1934), whereas that of K^+ may be considerably reduced if solution replacement is sufficiently rapid. Thus Johnston and Hoagland (1929) investigated the effect of concentration of potassium on growth of tomato in water culture in relation to different rates of solution renewal. 1·0 m. eq./L. K^+ (39 p.p.m.) was adequate at a renewal rate of 1·2 ml./minute/plant, but 0·1 m. eq./L. K^+ (3·9 p.p.m.) was inadequate ; equal growth was obtained for a rate of 8 ml./minute/plant for a concentration of 5 p.p.m. K and for a rate of 1·2 ml./minute at 39 p.p.m. Parker and Pierre (1928) found there was little difference between concentration of 0·125-0·625 m. eq./L. K^+ (5 and 25 p.p.m.) for the growth of single maize and soya bean plants in 7·5 L. water cultures frequently aerated and in which the potassium concentration was restored twice daily. Bartholomew and Janssen (1929) decided that the following potassium concentrations were

optimum for the growth of various plants in water cultures renewed twice daily with 2 plants per culture. Oat and cotton 2 p.p.m. ; clover, lucerne 0·5 p.p.m. ; Soya bean 2·5 p.p.m. ; Sudan grass 3 p.p.m. Higher concentrations led only to luxury consumption without additional growth. Later, however, Janssen and Bartholomew (1930) concluded that 0·25 m. eq. K^+ (10 p.p.m.) was not adequate for soya bean in sand or water cultures, or for oat in sand cultures, grown with continuous solution renewal. Tyner (1935) concluded that the minimum levels of potassium for clover and buckwheat in sand culture were 2 and 3 p.p.m. when the solution was renewed at the rate of 2 L./day. The nitrogen level was, however, low (about 4 m. eq./L.). Wall (1940) found that when solution was renewed at $3\frac{1}{2}$ L./day in sand culture with tomato, visual deficiency effects appeared in 14, 20 and 27 days for potassium concentration of 0·08 m. eq. (2·5 p.p.m.), 0·16 m. eq. (5 p.p.m.) to 0·3 m. eq. (11 p.p.m.) and 0·6 m. eq. (22 p.p.m.) respectively and were not seen at 1·2 m. eq. (45 p.p.m.), which was probably about the optimum. Ahlgren (1941) in similar experiments with white clover found that visual deficiency effects persisted with 0·4 m. eq./L. (16 p.p.m.) when solution was renewed at 1 L./day and only persisted at 0·1 m. eq./L. (4 p.p.m.) for a renewal rate of 4 L./day, but yields of dry weight were continuously increased up to 6·5 m. eq./L. (256 p.p.m.) for both rates of solution renewal. The deficiency or threshold level for potassium in intermittently watered sand cultures is about 0·75 m. eq. (30 p.p.m.) for potato, but growth may be increased by additional amounts up to probably 5 m. eq. (200 p.p.m. approximately) according to E. W. Jones (1948 *et seq.*, unpublished work). Mullison and Mullison (1942) regarded 1 m.eq./L. (39 p.p.m.) K^+ as optimal for barley under similar conditions. The potassium levels required by fruit trees in culture may vary considerably. Davidson and Blake (1937) found that visual symptoms of deficiency in peach trees in sand cultures appeared at 0·05 m. eq. (2 p.p.m.), but not at 0·25 m. eq. (10 p.p.m.) in agreement with Cullinan and Batjer (1943). Haas (1932 a) found that these levels were inadequate for citrus in water cultures, but Chapman *et al.* (1947) did not observe deficiency effects at 7 p.p.m. K^+ when large citrus trees were grown in large-scale water cultures, and only slight symptoms at 1 p.p.m. K^+. Batjer and Degman (1940) found that growth of apple trees in sand culture was reduced below 1·5 m. eq. (60 p.p.m.) K^+, and Cullinan and Batjer (1943) concluded that, in contrast to peach trees, apple trees in sand culture required up to 3 m. eq. K (117 p.p.m.).

Potassium requirements especially may be modified by light intensity and are generally lower during the summer according to Johnston and Hoagland (1929) and Turner and Henry (1939). Tolerance to high levels of potassium is often great and concentrations below 10 m. eq./L. do not usually cause abnormal effects unless calcium or magnesium are inadequate. Chapman and Liebig (1940) found that citrus was uninjured by 14 m.eq./L. K^+ (i.e. 546 p.p.m.) but injury occurred at 26 m. eq. (1,000 p.p.m.). The nitrogen level may also be an important factor in determining potassium requirements or the response of plants to different potassium levels. This is shown in the experiments of Richards and Templeman (1936), Gregory and Sen (1937) and further discussed by Gregory (1937).

Similar differences apply to phosphorus, but threshold concentration may be as low as ·04 m. eq./L. PO_4''' (0·4 p.p.m. P) for soya bean and maize according to Parker (1927) and Parker and Pierre (1928), and for citrus according to Chapman and Liebig (1940), and only 0·1 m. eq. or less (1 p.p.m.) for oat according to Behrens (1928) and Teakle (1929). Similar low thresholds were established by Tidmore (1929) for maize, sorghum and tomato and at somewhat higher levels by Sommer (1936), Cullinan and Batjer (1943) and Houghland (1947) for cotton, wheat, tomato, peach, apple, pea and potato. Hoagland and Martin (1923) and Kalin (1943), however, concluded that 1-1·5 m. eq. (10-15 p.p.m.) was necessary for tomato and barley. As might be expected the levels of other nutrient elements modifies the requirement for any one and the range given here can be probably interpreted on that basis.

Experience at Long Ashton has shown that the incidence of phosphorus deficiency may be determined by potassium and iron levels well within the range normally associated with freedom from toxicity.

A striking example of altered requirements for nitrogen is found in the results of Chapman and Liebig (1940). Citrus grew well in large capacity water cultures stirred by aeration

when the nitrogen level was between ·1 and 30 m. eq. (1·4 and 400 p.p.m.), but showed nitrogen deficiency in sand cultures until about seven times the lower concentration was applied. Cullinan and Batjer (1943) found that apple trees required 2-3 times as much nitrogen as peaches in sand culture.

It is obvious that no general rule can be deduced regarding either the optimum or threshold levels of all the nutrient elements. Woodman (1939 b) (1940 a, b) (1941 a, b) (1942) (1943 a, b, c) (1944 a, b) has investigated the effects of varied levels of N, P. K, Ca and Mg on several vegetables in sand culture and has applied statistical analysis to the data. From these experiments he deduced the optimum levels of various nutrient elements and found wide differences for many crops. Since, however, many of these experiments (which employed relatively pure reagents) did not include all the known micronutrients the validity of some of the conclusions may need re-examination, and in the opinion of the writer many of the levels were unduly low.

The well-used expression " nutrient balance " is an indication of both the latitude permissible for, and the modifying influence due to changes in concentration of each of the separate elements present in the solution. To discuss in detail the work done to elucidate problems of nutrient balance or interrelationships is clearly impossible here. It would lead the trend of this communication into the field of results rather than methods. The very recognition of this limitation, however, serves to emphasise the great importance of specifying nutrient composition.

Johnston and Hoagland (1929) first drew attention to the distinction necessary between concentration and total supply of a nutrient element. Arnon and Hoagland (1940) gave some data on the uptake of nutrients by tomato plants in relation to supply and volume of nutrient solution. The aggregate absorption of K^+, Mg^{++}, Ca^{++}, H_2PO_4' and NO_3' by a single tomato plant over a period of 5 months was 2,614 m. eq. 1 L. of nutrient provided 33 m. eq. of these ions and consequently 80 L. or so of solution were required to supply just the amount absorbed, assuming that the uptake was in the same proportion as the solution composition. The culture tanks provided a volume of 20 L. per plant and hence a minimum of 4 complete renewals at the concentration used would have been required. A detailed discussion of the problems of nutrient balance is given by Hoagland and Arnon (1941) and also by Beckenbach *et al.* (1936), Arnon and Hoagland (1940) (1944), Robbins (1946) and many others. The early controversy as to the best nutrient concentration, Brenchley (1916), Stiles (1915) (1916), is now of historical interest, since it is recognised that solution volume, rate or frequency of renewal, presence or absence of a solid medium, plant density, provision of stirring and nature of the plants all determine the minimum requirements for unimpaired growth. What is more important is to provide an adequate supply of *all* the known nutrients at concentrations as low as are compatible with adequate rate of absorption in relation to the containers, frequency of replacements, stability of pH and ease of diffusion around roots. This is especially important, as the number of important nutrient interactions is being continually extended.

It is evident that a great many compounds have been used to supply the minimum 6 macronutrient elements, namely, calcium, magnesium, potassium, nitrogen, phosphorus and sulphur. Livingstone and Tottingham (1918) pointed out that these elements can be supplied in 6 different combinations of 3 salts to prepare 3-salt solutions similar to those of Shive (1915 a, b). These 6 combinations were denoted by the prefix I-VI attached to Shive's solutions and were as follows :—

I	II	III	IV	V	VI
$Ca(NO_3)_2$	$Ca(NO_3)_2$	$Ca(H_2PO_4)_2$	$Ca(H_2PO_4)_2$	$CaSO_4$	$CaSO_4$
KH_2PO_4	K_2SO_4	KNO_3	K_2SO_4	KNO_3	KH_2PO_4
$MgSO_4$	$Mg(H_2PO_4)_2$	$MgSO_4$	$Mg(NO_3)_2$	$Mg(H_2PO_4)_2$	$Mg(NO_3)_2$

The choice of which to use is partly a matter of convenience and is partly determined by the fact that any particular element is linked to an equivalent amount of one of the others, and thus a high concentration of nitrogen must introduce a high concentration of one of the 3 metal ions and vice versa. There does not seem to be any theoretical reason to distinguish

between the 6 types as it is assumed that ionic equilibrium is attained irrespective of the initial combination of the anions and cations. By suitable combination of more than one salt of the same element, almost unlimited range of composition can be obtained (pp. 167-168).

No especial benefit attaches to the use of 3-salt solutions in the opinion of the writer. They suffer from a number of limitations including :—lack of flexibility, difficulty of replacement of one element by another in deficiency studies, and omission of sodium. On the other hand, when micronutrient deficiency problems are concerned, the use of the minimum of nutrient reagents that are well characterised and easily purified is an important requisite unless there are special reasons to the contrary. A 4-salt type of nutrient based on calcium and potassium nitrates, sodium phosphate and magnesium sulphate, with or without the inclusion of a limited amount of an ammonium salt, would appear to fulfil all the requirements of flexibility, simplicity, ease of purification and inclusion of sodium in accordance with the principles described in this and the following sections. The composition of such a nutrient solution is well illustrated by those of Arnon (1938), Arnon and Hoagland (1940) and the Long Ashton solution given in Part Two.

Osmotic Pressure, Salt Concentration and Sodium Content of Nutrient Solutions

Osmotic Pressure

Great attention was paid in early experiments to the measurement and determination of nutrient solutions in terms of their osmotic pressure. This property of nutrient solutions was given especial prominence in relation to total nutrient concentrations in the work of Tottingham (1914), Shive (1915 a, b) (1918 b), McCall (1916 a, b), Hoagland (1919), Reed and Haas (1924 a). Tottingham prepared Knop's solution and found the maximum concentration that did not precipitate during 14 days to be 2·2% (with K_2HPO_4) and 2·8% (with KH_2PO_4). He then prepared three nutrient series having total salt concentrations of 2%, 0·6% and 0·01% which corresponded to osmotic pressures of 8·15, 2·5 and 0·05 atmospheres. The total osmotic pressure was divided into ten equal parts corresponding to ten sets each of molar fractions of the four principal nutrient salts. Thus the total osmotic pressure was produced from the sum of at least one-tenth and not more than seven-tenths of the partial osmotic pressure from each of the 4-salt components. The 84 combinations thus produced were arranged as a set of triangular diagrams for each phosphorus level and thus could be represented by a pyramid. Reproduction here of this now classical work is unnecessary, as it can be seen in Miller's " Plant Physiology " and in the original paper ; (Tottingham 1914). Tottingham used wheat and concluded that 2·5 atmospheres O.P. was optimal. The " best " composition was judged to be as follows :—

	Molar Fraction	O.P.
KH_2PO_4 (T) (Triangle)	·0130	3 tenths
KNO_3 (R) (Row)	·0049	1 tenth
$Ca(NO_3)_2$ (C) (Column)	·0144	4 tenths
$MgSO_4$ (D) (Difference)	·0145	2 tenths
		10 tenths

and the code assigned was thus $T_3R_1C_4$ (Table 19 A). Shive (1915 a) carried out similar work, but introduced the idea of using the minimum of three principal nutrient salts. When each was made to contribute from one to eight-tenths of the total osmotic pressure a series of 36 types were produced. Each series was tested at total osmotic pressures of 0·1, 1·75 and 4·0 atmospheres, and 1·75 atmospheres was regarded as optimal. Shive (1918 b) deduced that the "best" solution for buckwheat in small volume water culture (Table 19 A) was that designated as R_4C_3 and was comprised as follows :—

	Molar fraction	O.P.
$Ca(NO_3)_2$	·0052	2 tenths
KH_2PO_4	·0144	4 tenths
$MgSO_4$	·0200	4 tenths

This solution was then compared, at the three total osmotic pressure values given, with Tottingham's best $T_3R_1C_4$ and Knop's original ratios each at the same osmotic concentrations. Shive's solution with a total osmotic pressure of 1·75 atmospheres gave the best yields and the Shive's solution was also relatively superior at each concentration tested. Similar experiments on sand cultures were made by McCall (1916 a, b). He tested solutions of several total osmotic pressures and also concluded that 1·4-1·8 atmospheres was optimal. The conclusions drawn from these experiments and others made during the same period were largely invalidated, however, since it was not possible to distinguish between the effects of variation in concentrations of nitrogen and calcium, or of potassium and phosphorus, etc. An early attempt to elucidate the effect of individual ions in 3-salt solutions was made by Gregory (1928 b), who re-examined data from an experiment by Johnston (1924) (p. 167). Another criticism is that as the micronutrients were usually omitted the effects of impurities in the major salts may have outweighed the effects inferred.

Beckenbach *et al.* (1936) adopted a new approach to the problem of trying to evaluate solutions on the triangular system. They selected four of Shive's solutions prepared with an osmotic pressure of 0·5 atmosphere and supplied them by continuous renewal at 1 L/day to maize in water cultures. The four solutions chosen were those corresponding to each apex and the central point of Shive's triangle. For *each* solution the concentrations of anions or of cations were then varied independently by suitable combination of nine sources of the three principal ion pairs, whilst the ions of opposite sign were kept constant. For example, the solution represented by the code R_3C_3 was prepared in two ways. First, so that the nitrate, phosphate and sulphate components were present as required by the standard formula given by Shive, whilst the levels of calcium, potassium and magnesium were varied by arranging that each contributed from one to eight-tenths of the total osmotic pressure due to all cations, and second, so that the same variation was applied to the anions. In this way the covariable effect of pairs of ions was eliminated from the experiment. They concluded from a statistical analysis of the results that the best comparison was that shown by the following ratio of osmotic pressure fractions (as tenths) :—

N	:	P	:	S	: :	8	:	1	:	1
Ca	:	K	:	Mg	: :	3	:	3	:	4

Further discussion of the use of triangular and statistical approaches to these problems is reserved for the Section on lay-out of experiments.

Hoagland (1919) tested his solution given in Table 19 at 0·07, 0·1, 0·25, 0·32, 0·78, 1·45 and 2·00 atmospheres osmotic pressure in sand and water cultures with a relatively large volume of 5 gallons for only 4-5 plants. Concentrations equivalent to ·78 and 1·45 atmospheres were optimal and gave equal yields in sand culture ; osmotic concentrations of 0·1 and 0·25 atmospheres were definitely low. For the water culture the optimum range was lower, being 0·32-0·78 atmospheres. Hoagland was the first to point out that earlier work had failed to distinguish between concentration and total amount of nutrients in a solution, and this concept was elaborated by Johnston and Hoagland (1929). Hoagland (1919) defined an optimum nutrient solution as " the minimum concentration which gave maximum yield and beyond which there was no further improvement."

Reed and Haas (1924 a) tested their standard solution (Table 19 B) with *Citrus* sp. in water cultures at overall concentrations of 0·25, 0·5, 1, 1·5, 2 and 2·5 times the standard level. These solutions had osmotic pressures ranging from 0·53-1·6 atmospheres and an optimum at 1·2 atmospheres corresponding to 1·5 times the standard concentration. Wadleigh *et al.* (1937) tested the growth of maize in sand cultures at osmotic pressures of 0·1, 0·5, 1·5 and 3·0 atmospheres. Best growth occurred at 0·5 atmospheres. In a general consideration of the optimum solution concentration Livingstone (1942) recommended a value of 0·02 M, equivalent to about 1·0 atmosphere, as satisfactory, and suggested that 0·06 M (2·5 atmospheres) was definitely an upper limit and that 0·01 M was probably adequate when continuous renewal methods were applied. Hoagland (1944) also regarded 2 atmospheres as a probable upper limit. Robbins (1946) recommended total concentrations for sand culture similar to those of Livingstone (1942).

Salt Tolerance

There is some evidence that concentrations even slightly in excess of 1 atmosphere may be deleterious. Thus Robbins (1937) found that solutions having concentrations equivalent to 1·7 atmosphere pressure or more caused blossom end rot in tomatoes due to loss of water from the fruit. Ahi and Powers (1938) found that the threshold tolerance to various salts for lucerne were 17 m.eq./L sodium chloride, 20 m.eq./L sodium sulphate, and 6-7 m.eq./L for sodium bicarbonate. Similarly, Heller *et al.* (1940) found that tomato plants tolerated 600-800 p.p.m. of sodium chloride ; 1,600 p.p.m. was markedly toxic. These levels corresponded to 10-14 and 28 m.eq./L respectively and to approximately 0·5 and 1·2 atmospheres. Eaton (1941 b) regarded 7·5-10 m.eq./L of added calcium, magnesium and sodium salts (about 0·3-0·5 atmosphere each) as about the threshold for tomato in water culture. Hayward and Spur (1944) tested the effects of osmotic pressures of 0·5, 1·5, 2·5, 3·5 and 4·5 atmospheres on the growth of flax in sand culture. Yields were reduced at all levels above the lowest. Similar results were obtained for beans in water culture by Gauch and Wadleigh (1945). On the other hand, Hayward and Long (1941 a) found that tomatoes grew better in sand culture at a solution concentration of 1·5 atmospheres than at 0·5 atmosphere, and Hayward and Long (1941 b) found that peach trees in sand culture were benefited by additional chloride or sulphate up to 0·75 atmosphere (10-15 m.eq./L) and only slightly depressed at a concentration equivalent to approximately 2 atmospheres. Eaton (1942 a) concluded that tomatoes grew best in sand cultures in the presence of 2·5-10 m.eq./L of sodium chloride or sulphate, whilst maize tolerated only 2·5 m.eq./L sulphate or 4 m.eq./L chloride.

Several other experiments on the effects of relatively high salt concentration on growth have been recorded by Nightingale and Farnham (1936), Wall and Hartman (1942), Gauch and Magistad (1943), Hayward and Long (1941 a) (1943), Hayward and Spur (1944), Magistad *et al.* (1943), Gauch and Wadleigh (1945). Nightingale and Farnham found marked changes in anatomical development and chemical composition of peas grown with solutions of different osmotic pressures. Hayward and Long (1941 a) also found that tomato plants responded differently in anthesis and succulence according to the osmotic pressure of the culture solutions.

Different species vary somewhat in sensitivity to high salt concentration. Magistad *et al.* (1943) reviewed this aspect and in their own experiments they found that dwarf beans were particularly sensitive, and carrot and beet progressively less so. Relative yields for these crops at different osmotic pressures were as follows :—

Magistad *et al.* (1943)

	0·4 ats. (Basal)	2·4 ats.	4·4 ats.
Bean	100%	50%	Nil
Carrot	100%	70%	18%
Beet	100%	95%	70%

They found also that growth depressions were almost directly proportional to the *increase in osmotic pressure* and *not* to the concentration as m.eq./L. That depression effects were due mainly to osmotic pressure was also concluded by Hayward and Long (1943) and by Gauch and Wadleigh (1945). Some salts, however, are more toxic than others, sodium bicarbonate as compared with chloride and sulphate is particularly so.

Ahi and Powers (1938), Heller *et al.* (1940), and Wall and Hartman (1942) established the relative order of toxicity of certain salts at 1,000 p.p.m. concentration to growth of tomatoes to be as follows :—$NaHCO_3$> $MgCl_2$> $CaCl_2$> Na_2SO_4> $MgSO_4$ and this corresponds roughly to the number of ions produced in solution (except possibly for $NaHCO_3$). Climatic conditions also modify salt tolerance. Wall and Hartman (1942) found that tolerance was much greater at low temperatures. Magistad *et al.* (1943) found that tolerance of onions was especially sensitive to the effects of climate. Hot dry climates caused great injury at high salt concentrations, but growth in similar solutions was almost normal in a cool climate. Beet showed a similar but less marked response.

Examination of Tables 19 A & B indicates that osmotic pressures of nutrient solutions have been greatly reduced from the values commonly used in earlier experiments. The range of osmotic pressure of modern nutrient solutions appears to lie in the region of 0·4-1·0 atmosphere. The upper limit is more applicable to the method of intermittent application and relatively limited culture volume whilst the lower limit is more suited to continuous renewal type of cultures. The problem of physiological availability and concentration is discussed by Hoagland and Arnon (1941) in a critical contribution to the subject.

Sodium Content.

It may be concluded that in general the osmotic pressure of the nutrient solution should not exceed 1-1·5 atmosphere. The injurious effects so produced appear to be osmotic effects rather than due to sodium ions, although in most experiments the latter were increased with increased osmotic pressure. Magistad *et al.* (1943) concluded that the sodium ion as such was not the main injurious factor in effects associated with increased osmotic pressure. Hoagland and Snyder (1933), however, found that sodium in excess of 100 p.p.m. caused injury to strawberries in water culture. In unpublished experiments by the writer, celery was also injured by only moderately high concentrations of sodium (8 m.eq./L) especially when calcium was deficient. Sugar beet tolerated high concentrations of sodium chloride, whereas sodium sulphate (11 m.eq./L) but not potassium sulphate, induced calcium deficiency. High concentrations of sodium nitrate induced symptoms resembling calcium deficiency in cauliflowers.

Numerous experiments, however, indicate that sodium is beneficial under certain conditions or to special crops. The ability of sodium to mitigate potassium deficiency was demonstrated by Hellriegel and Wilfarth about 1898 (see Dorph-Peterson and Steenbjerg 1950). The partial substitution by sodium for potassium was later studied by Hartwell *et al.* (1907) and Hartwell and Pember (1908) using cereals in water culture, and by many others since.

Lehr (1941) (1942) (1947) made a detailed study of the role of sodium in the growth of sugar beet from the physiological and practical agricultural aspects. He concluded (1942) that sodium is markedly beneficial to beet grown in ion-exchange type cultures both in the presence and more especially in the absence of sufficient potassium. Unpublished work by the writer indicated also that potassium deficiency symptoms in beet grown in sand culture could be practically eliminated by extra sodium (8-11 m.eq./L). Total lack of sodium even with adequate potassium was unfavourable and plants tended to wilt. Absence of both elements caused acute and specific deficiency effects distinct from those normally associated with potassium deficiency in the presence of 1-3 m.eq./L. of sodium.

Lehr (1942, p. 409) inferred that sodium fulfilled an " almost independent function " in beet in addition to its role as a partial substitute for potassium. This view was then qualified by the statement (*loc. cit.*) that " in the equilibrium of cations, however, the decisive factor is the exact proportions rather than the absolute quantities." Holt and Volk (1945) similarly regarded sodium as specifically beneficial to oat and wheat as well as to sugar beet independently of the level of potassium nutrition, and found that all crops showed some beneficial response to sodium. Mullison and Mullison (1942) held the same opinion regarding the effect of sodium on barley. Eaton (1936) recommended inclusion of sodium up to 2 m.eq./L in the nutrient solution, especially if tomatoes were to be grown. Shive and Robbins (1942) also suggested that the inclusion of sodium might be desirable and stated that formula II, p. 86, where sodium ions were included, gave excellent results with tomato, cotton, tobacco and radish in particular. Robbins (1946, however, made no special recommendation for the use of sodium salts. The view expressed by Lehr (1942, p.409) undoubtedly goes far to explain the effects of sodium, and it was early recognised by Richards (1932) and by Richards and Templeman (1936), when they drew attention to the consistent discrepancies between observations by different workers on potassium deficiency symptoms and succulence in barley, and pointed out that variations in the ancillary ions, especially sodium, might well be responsible. Barley provides a good example of the complexity of

potassium-sodium (and -calcium) interrelationships as shown by the work of Richards and Shih (1940 a, b). These workers found that sodium level showed an outstanding and significant positive correlation with water content of barley plants grown in a factorial experiment where levels of sodium, potassium, calcium and phosphorus were all varied. Phosphorus level was next in importance and calcium least of all. Potassium level was not directly related to succulence, but exerted a complex interaction effect on the extent to which the other elements affected succulence.

Gregory (1937) summarised these and other results in a wider discussion of the profound effects of Na/K/Ca balance on succulence, visual symptoms, and carbohydrate and nitrogen metabolism. Mention must be made here also of the effect of rubidium on potassium nutrition studied by Richards (1941). Rubidium exerted a slightly beneficial effect on potassium-deficient barley when ammonium nitrogen and a high phosphorus level was given, but a specific toxicity could also be detected in all treatments.

It is clear that in any experiments on potassium deficiency the sodium content especially requires to be specified and carefully controlled or, if a variable, taken fully into account in analysing the results.

Tables 19 A & B show that many evidently successful nutrient solutions are devoid of sodium and it is reasonable to conclude that for most plants this element is not essential. There is, in fact, no evidence at all to justify the conclusion that sodium is an essential element in the terms of the criteria of essentiality proposed by Arnon and Stout (1939 a). It is equally important to recognise the frequently apparently beneficial effect of the element in culture work—sufficient to justify a preference for the 4-salt type of nutrient containing sodium such as the Long Ashton solution, and the undisputed value of sodium manuring for certain crops, especially beet, and the tolerance of others, e.g. barley. Lehr (1941) and Dorph-Peterson and Steenbjerg (1950) have clearly emphasised this distinction and reviewed the important literature to date.

The Source of Nitrogen in Nutrient Solutions

Nitrate and Ammonium Nitrogen

The most frequently used source of nitrogen in culture solutions is nitrate. Nitrate type nutrients produce satisfactory growth with many crops when certain requirements are fulfilled and, in general, seem to be superior to other sources. Although nitrate has been used from the earliest days, ammonia nitrogen was used in the early work of Sachs (1860), Hampe (1868), Mazé (1900) (1914) and Hutchinson and Miller (1909). The use of nitrite, ammonium and organic sources of nitrogen has been frequently adopted since then and will be outlined below, but the circumstances leading to satisfactory results seem more circumscribed and failures are common. The use of nitrate is so general that little comment seems necessary, but a few points may be mentioned in a general way in a comparative treatment of the use of nitrate and ammonium salts which are the two main forms of nitrogen.

Relation to pH. Solutions based on nitrate nitrogen tend to become more alkaline in contact with growing plants and may become physiologically "iron deficient" especially in water cultures. This entails more frequent additions of iron compounds and greater attention must be given to their nature. The rise in pH is due to differential uptake of nitrate and the principal cations; the latter (except the ammonium ion) are absorbed less rapidly and the pH increases. The uptake of nitrates, although it occurs readily under widely different conditions, is facilitated by slightly acid pH values of 4·5-6, whereas the uptake of ammonium nitrogen occurs more rapidly at somewhat higher pH values of 6-7, cf. Naftel (1931), Tiedjens and Robbins (1931), Tiedjens and Blake (1932), Clark (1933), Clark and Shive (1934), Davidson and Shive (1934), Pardoe (1935), Hoagland (1937), and Nightingale (1937). Nightingale (1934), and Clark and Shive (1934) concluded respectively that rates of uptake of nitrate and ammonium nitrogen were equal at pH 6·0 and pH 4·5-6·5. The relation between source of nitrogen and pH regulation is considered later.

The optimum pH of the nutrient also varies with the source of nitrogen. Nitrate usually gives best results at pH values suitable for maximum rate of uptake, and the same general rule applies to ammonium nitrogen, *loc. cit.*, and also according to Tiedjens (1934), Arnon (1937), Haas (1940) and Clarke (1941). The optima appear to be about 4·5-5 for nitrate and 6-6·5 or above for ammonium nitrogen.

Solution renewal. The relative value of nitrate and ammonium nitrogen and the region of the pH optima may vary according to whether the solutions are continuously renewed or are only changed at intervals. This is well illustrated by data of Pirschle (1931 b) below for yields of maize grown at different pH values.

Pirschle (1931 b)

Source of N		pH	3	4	5	6	7	8	9	
NH_4 type	Dry wt.	g.	0·4	0·9	3·4	5·7	7·3	7·6	2·8	Static culture
NO_3 type	Dry wt.	g.	7·6	11·8	10·5	10·5	9·9	4·7	3·9	Ditto
NH_4 type	Dry wt.	g.	0·5	8·1	11·4	11·4	8·5	7·6	3·4	Flowing culture
NO_3 type	Dry wt.	g.	0·6	9·2	10·3	11·2	7·6	8·7	8·3	Ditto

Yields of oat, maize, wheat, pea and buckwheat were increased (in some instances up to 20 times) by use of flowing cultures when ammonium nitrogen was given. The dependence of the relative effects of ammonium and nitrate nitrogen on the method of solution renewal is exemplified further in the experiments of Tiedjens (1934) and Tiedjens and Robbins (1931).

Season. The relative yields and optimum pH for these sources of nitrogen may vary with season, according to Pardoe (1935), Arnon (1937), and Robbins (1946), owing to change in duration and intensity of light in relation to the accumulation and mode of storage of these nitrogen sources. Arnon (1937) obtained the following data for dry weight of barley grown in water cultures.

Arnon (1937)

Source of N.	Season	pH 4	5	6	6·7
NH_4^+	Spring	0·3	0·7	1·3	0·9
	Autumn	0·5	1·05	1·1	1·15
NO_3'	Spring	0·5	1·9	2·0	1·8
	Autumn	0·8	2·1	1·7	1·6

The optimal pH values moved in opposite ways for the two nitrogen sources, with change of season. At pH 6, nitrate was superior to ammonium at all seasons from October till May and especially in mid-winter, but the effect was reversed slightly in June. Robbins (1946) advised omission of ammonium compounds when light intensities were low.

Aeration and micronutrient supply. Forced aeration may be less important in water cultures supplied with nitrate than when ammonium is used. Arnon (1937) has studied the problem as it affects the growth of barley in water culture and the inter-relationships of source of nitrogen, season, aeration and supply of micronutrients, particularly copper and manganese. Arnon found that barley was far more responsive to additions of these elements in the presence of ammonium nitrogen, and that additions of either of them practically offset the omission of aeration when this source of nitrogen was used. On the other hand, the requirements of molybdenum for some plants may be greatly reduced or nil in the presence of ammonium nitrogen, Mulder (1948 a). Similar conclusions were shown by Steinberg (1936) (1937) (1939) in work on *A. niger*. Recent work at Long Ashton has shown that molybdenum is still essential in the presence of ammonium compounds alone or with nitrate.

Nutrient uptake. The uptake of macronutrient ions is modified according to the source of nitrogen. In the presence of ammonium nitrogen the uptake of calcium and magnesium was depressed in experiments made by Jacobsen and Swanback (1933), Nightingale (1937) and McEvoy (1946). Hewitt (1947 b) found that kale, sugar beet and tomato grown in sand culture developed calcium deficiency in the presence of ammonium nitrogen.

Absorption of ammonium nitrogen is also more rapid than that of nitrate, although the relative rates also vary with species and age of the plant. Nitrate is usually absorbed in increasing amounts, and ammonium nitrogen in decreasing amounts, as age increases. This was shown by the work of Mazé (1900), Jones and Skinner (1927), Naftel (1931), Stahl and Shive (1933), Clark and Shive (1934), Pardoe (1935), Hoagland (1937), and Nightingale (1937).

Iron requirements. One of the differences between the use of the two sources of nitrogen lies in their effect on iron requirement and supply. In many instances the advantageous effects observed from the use of ammonium nitrogen could be attributed solely to the elimination of chlorosis, as was evident from the results of Jones (1920), and of Jones and Shive (1921 b), given here, for the effect of replacement of part of the nitrate by ammonium nitrogen.

Jones and Shive (1921 b)

Dry wt. of Wheat g.	Solution	Source of Iron	Remarks
1·44	Tottingham	$FePO_4$	Chlorotic
2·55	Modified Tottingham $(NH_4)_2HPO_4$ in place of KNO_3	,,	Green
3·97	Tottingham	$FeSO_4$	Green
1·23	Modified Tottingham	,,	Green

Barnette and Shive (1923) produced similar results with these two solutions and found that, whereas chlorosis always occurred in the Tottingham solution at pH 6·0 or above, plants grown in the modified solution were not chlorotic, in spite of being given only one-twentieth of the iron supply, and gave higher yields. Tiedjens and Robbins (1931) found that, whereas chlorosis occurred in many crops in sand cultures when supplied by continuous solution renewal with nitrate at pH 6 or higher, no chlorosis occurred at pH 8 with ammonium nitrogen and slightly greater yields were obtained. Tiedjens and Blake (1932) found similar results with apple trees in sand culture except that yields with nitrate still exceeded those with ammonium nitrogen in spite of freedom from chlorosis in the latter treatment. Similar results were also obtained by Davidson and Shive (1934) for peach trees and by Clark (1941) for strawberries grown in sand culture. The maximum yield with nitrate almost always exceeded that with ammonium nitrogen in spite of the effect on chlorosis and the use of a high rate of solution renewal. Thelin and Beaumont (1934) found that ammonium nitrogen caused less chlorosis than nitrate in wheat and rice grown in water culture. Asana (1945) found that the presence together of ammonium and nitrate nitrogen prevented chlorosis in rice grown in water culture and resulted in greater yields than were obtained with either alone. A similar result was obtained by van der Veen (1940) with *Coffea arabica* and *C. robusta* in water cultures. A ratio of 5 m.eq. NO_3' to 1 m.eq. NH_4^+ gave the best growth in both species. Sideris *et al.* (1943) found that pineapples grown in water culture derived sufficient available iron from the impurities in reagent salts when ammonium nitrogen was used, but required additional iron in the presence of nitrate.

Relative tolerance and effect on growth. Several investigators have compared the effects of the two sources of nitrogen on yields or other growth responses. Mazé (1914) found that injury to maize in water culture due to ammonium sulphate was prevented by giving calcium carbonate, and Hewitt (unpublished) found that calcium carbonate mixed in the sand also eliminated ammonium toxicity ; but Beaumont *et al.* (1933) were unable to prevent toxicity to grasses and clovers by this method in water culture. Wolkoff (1918) found that ammonium

sulphate injured young soya bean plants in sand culture. Brenchley and Warington (1927) found that a solution containing ammonium sulphate and a low level of calcium was markedly unfavourable for broad bean. Beaumont (1930) stated that ammonium nitrogen was toxic to tobacco in water cultures at concentrations above 6 p.p.m. N. Thelin and Beaumont (1934) found that ammonium compounds were more toxic to rice than nitrate, in spite of less chlorosis. Rice was, however, more tolerant than wheat, and thrived better on ammonium nitrogen in early stages, but plants given nitrate grew better when more advanced. Pirschle (1931 a, b) found that yields of pea and oat were always greater with nitrate nitrogen when grown in sand or water cultures with intermittent renewal of solution. Similar results were obtained for wheat, flax, buckwheat, *Tradescantia*, blue lupin and white mustard except at pH 7-8 and for barley at pH 9, where the ammonium nitrogen cultures were superior. Rice, however, gave better yields with ammonium nitrogen at most pH levels from 5 upwards, but the maximum yield at pH 3 with nitrate still exceeded all yields obtained with ammonium nitrogen. Pirschle (1931 a, b) also found that, with continuous solution renewal, yields with nitrate still exceeded those with ammonium nitrogen for rape, oat, barley, rye, *Ricinus*, broad bean and buckwheat, but the position was reversed for wheat at pH 5-7, soya bean at pH 5-7, rice at pH 6-8 (or 5-7), flax at pH 6-7 and pea at pH 6-7. Alben *et al.* (1942) found that the presence of ammonium sulphate in Eaton's (1936) solution resulted in a pH of 3·0 when used for pecan trees, with consequent injury.

Many crops appear to grow well when the nutrient contains both nitrate and ammonium nitrogen, as cited above, cf. also Davidson (1928), Pardoe (1935), Shive and Robbins (1942), McEvoy (1946), Robbins (1946), and this combination is the basis of many highly successful nutrient solutions, particularly effective in water cultures as well as in sand cultures, as for example that of Arnon (1938). In a few instances ammonium nitrogen solutions appear as satisfactory as those containing nitrate as, for example, was found by Addoms (1937) for growth of loblolly pine, and Sideris and Krauss (1937), who showed that the growth curves of pineapple plants grown in water cultures, with only 28 p.p.m. N., were almost identical for either source of nitrogen.

Lewis (1936) considered that ammonium nitrogen was superior to nitrate for the growth of rye grass in sand cultures containing bentonite, which can hold cations in an absorbed state, and also 4% of calcium carbonate. Nostitz (1925) also grew oat and barley in sand cultures, where the nitrogen was present as the ammonium ion adsorbed on Permutit materials and obtained better growth than with all the cations in solution. Tiedjens and Robbins (1931) found that ammonium hydroxide was a good source of nitrogen for many crops in sand culture. It was superior to ammonium sulphate or calcium nitrate, and no toxicity was observed up to 0·0042 M. NH_4OH. Ammonium citrate has been tested at Long Ashton. It practically eliminated ammonia toxicity to cauliflowers and minimised pH changes.

Further evidence is given by Nightingale (1937), and in an extensive review by Pardoe (1935) who concluded that the subject is highly controversial owing to the interaction of the numerous factors already considered. He concluded that nitrate was usually the superior source of nitrogen, but that rice surpasses all graminaceous plants in its tolerance to ammonium nitrogen. Maize is " indifferent " and barley, oat and rye, legumes and most other crops " prefer " nitrate.

The various points arising from this discussion may be summarised as follows :—

1. Nitrate results in increase in pH, is absorbed more readily at lower pH values and gives better growth at lower pH values than ammonium nitrogen, which may be toxic.
2. Nitrate may require higher concentrations of iron compounds and also of molybdenum, but possibly less copper and manganese, than ammonium nitrogen, which also depresses uptake of calcium.
3. Ammonium nitrogen may increase the need for aeration of the culture solution. The relative merits of solutions based on nitrate or ammonium nitrogen are influenced by the age and species of the culture plant, the season, the rate of renewal and the pH of the culture solution.
4. These factors may affect growth less critically when nitrate is used.

TABLE 19A

Composition of Nutrient Solutions Selected to Show Historical Development and Typical Modern Examples

Left-hand columns : concentration as milligram equivalents per litre—m.eq./L.
Right-hand columns : concentration as parts per million—p.p.m.
Only one column : concentration as parts per million—p.p.m.
(Approximated data)

Reference	O.P. (Ats.)	pH	Ca		Mg		Na		K		N as NH_4		N as NO_3		P as PO_4		S as SO_4		Cl		Fe	Source of Fe	Mn	Cu	Zn	B	Mo	Remarks
Sachs (1860)			13·3	266	4	48	4·1	95	9·9	386			9·9	139	7·5	78	9·8	177	4·1	145	trace	$FeSO_4$	Trace $MnSo_4$					First published standard formula ; prepared with KNO_3, $Ca_3(PO_4)_2$, $CaSO_4$ $MgSO_4$ NaCl
Knop (1861)			8	160	2	24			4·7-6·1	184-240			12	170	2·1–6·4	21–66	2	32			130	$FePO_4$ trace $FePO_4$ (up to 0·1 g Knop 1861)						An efficient 4-salt type basal solution prepared from $Ca(NO_3)_2$ KNO_3 KH_2PO_4 $MgSO_4$
Knop (1865)			12·2	244	2	24			4·3	168			15·6	206	5·5	57	2	32			trace							
Birner and Lucanus (1866)			26·6	532	12·2	147			10·8	422			26·6	372	57·5	594	12·2	195			52	$Fe_3(PO_4)_2$						High phosphorus level, high iron level as sparingly soluble $Fe_3(PO_4)_2$ and relatively concentrated.
Hampe (1868)			1·7	34	1·7	20			3·35	127	6·7	94			20·1	208	1·7	27	1·7	60	trace	$FePO_4$						Nitrogen all as ammonia, high phosphate and low calcium content.
Fellens (1882)			25·6	512	9·8	118	5·4	124	9	351			30·8	431	11·4	118	9·8	158	5·4	182	trace	$FePO_4$						High calcium nitrate level and relatively concentrated.
Schimper (1890)		7·3	25·8	516	8·8	106	5·2	118	11·4	446			31·1	435	9·3	96	8·8	141	5·2	175	6	$FeCl_3$						A relatively concentrated solution. Phosphate supplied as K_3PO_4.
Pfeifer (1897) (1905)			9·8	196	1·6	19			5·6	218			12·5	175	4·4	45	1·6	26	1·3	47	trace	$FePO_4$						A balanced nutrient solution containing nitrate type.
Detmer (1898)			12·2	244	2	24			6·2	242			12·2	171	4·2	43	2	32	3·3	117	trace	$FePO_4$						An alkaline solution prepared with K_2HPO_4 or $Ca(NO_3)_2$.
Mazé (1900)		Probably >7·8	Solid $CaCO_3$ 2 g/L.		1·6	19	11·8	268	7·4	290			11·8	165	22·1	228	2·9	47	1·6	57	37	$FeSO_4$	44		trace			An early attempt to evaluate the relative micronutrient requirements but too high in manganese. Also included trace of K_2SiO_3.
Bruch (1902)		7·0	2·9	58	2	24	·14	3·3	7·9	307			5	70	4·4	45	4·9	79	0·14	5	3	$FeCl_3$						Rather low in calcium.
Van der Crone (1902–1904) Given by Tottingham (1944)			7·7	154	2	24			9·9	386			9·9	139	7·8	80	4·9	79			84	$Fe_3(PO_4)_2$						An efficient nutrient solution still widely used especially for water culture owing to relative freedom from iron precipitation by use of organic source of phosphorus available.
Hartwell, Wheeler and Pember (1907)			3·5	70	0·8	10			0·8	31			3	42	1·5	15	0·8	13	0·8	28	trace	$FePO_4$						A relatively dilute type of solution low in nitrate.
Schreiner and Skinner (1910)			1·3	26			27·8	639	6	234			27·8	389	4	41	6	96			trace	$FePO_4$						Low in calcium and apparently limited in micronutrients.
Tottingham (1944) T_3 R_1 C_4	2·5		28·8	576	29	348			13	507			28·8	403	39	400	20	464			trace	$FePO_4$						Tottingham's best, selected from 24 solutions tested in a sand culture at a pressure of 2·5 at. and using the salts present in Knop's solution. Relatively concentrated.
Mazé (1915)		Probably >7·8	Solid $CaCO_3$ 1·5 g/L.		0·8	10	6	137	5·7	223			6	84	8·6	88	1·3	21			10	$FeSO_4$	5·5		9·7	0·48		Probably the first attempt to include several known ato. only micronutrients at moderate although excessive concentrations ; included 0·029g./L. K_2SiO_3.
Shive (1915 a) R_4 C_2	1·75		10·4	208	40	481			14·4	562			10·4	148	43·2	448	40	640			trace	$FePO_4$						Systematic study of nutrient types based on minimum of 3 basic elements. Relatively concentrated by modern standards.
McCall (1916 a)	1·75		36·4	728	10	121			7·2	281			36·4	510	21·6	224	10	160			trace	$FePO_4$						Nutrient balance.
Hartwell and Pember (1918)			3·1	62	1·6	19			0·8	31	1	14	3	42	0·18	2	1·6	26			trace	$Fe(NO_3)_3$						Low phosphate level used in experiments on aluminum toxicity.
Livingstone and Tottingham (1918)			4·8	97	10	121			28·8	1124			28·8	103	14·5	149	10	160			trace	$FePO_4$						Ca and PO_4 supplied as $CaH_4P_2O_8$, high K level.
Hoagland (1919)	0·78	6·8	10	200	7·8	99	·5	12	7·1	284			11·4	158	4·1	44	7·8	125	0·5	18	as required	Citrate or tartrate						A new type of solution based on the composition of the soil solution, and supplying iron as citrate or tartrate.
Jones and Shive (1921 a, b)	1·5		14·6	292	14·2	172			2·6	102	2·8	39	14·6	201	6·3	65	14·2	227			0·83	$FeSO_4$ compared with $FePO_4$						Early work on relation of iron deficiency to source of N and pH
Tarr and Noble (1922)		4–7·6	5	100	10	121	15	345	8·2	321			18·2	254	25	255	10	160	5	177	trace	$FePO_4$						Use of potassium phthallate and phosphoric acid to control pH.
Crone's solution as used at Rothamsted (Courtesy W. E. Brenchley)			10·6	212	4	18			9·9	386			9·9	139	7·8	80	9·8	157			84	$Fe_3(PO_4)_2$	0·25			0·2		Of especial value in water culture for plants liable to chlorosis.
Rothamsted solutions (Courtesy W. E. Brenchley)		4·5 5·5 6·2	5·8 5·8 5·8	116 116 116	4 4 4	48 48 48			14·0 14·4 15·2	546 562 593			9·9 9·9 9·9	139 139 139	11·1 11·2 11·3	115 116 117	9·8 9·8 9·8	157 157 157	(0·5 (0·5 (0·5 (absent when Ferric Citrate is used)	17) 17) 17)	8 8 8	$FeCl_3$ or Ferric Citrate	0·25 0·25 0·25			0·2 0·2 0·2		pH range obtained by variation of K_2HPO_4 — KH_2PO_4 ratio. Phosphate level relatively high.
McLean and Gilbert (1926) (i)		4·5	0·2	4	0·32	4			2	78	2	28	4	56	0·96	10	0·2	3·2			1·5	$Fe(NO_3)_3$						Magnesium and phosphorus given as $MgH_4P_2O_8$ and lower calcium. Calcium and phosphorus given as $CaH_4P_2O_8$. A little but well-balanced solution rather low in K.
,, ,, (ii)			3·1	63	1·6	19			0·8	31	1	14	4	56	0·4	4	1·6	26	0·8	28	0·22	$Fe(NO_3)_3$						
Zinzadze (1926) (i)	0·96	5·0	19·4	388	4	48			9·9	386	4·7	66	6·4	90	13·6	140	9·8	157	8·2	287	45	$Fe_2(SO_4)_3$						Early attempts to prepare solutions of constant pH values.
,, ,, (ii)	0·90	5·0	16·9	338	4	48			9·9	386	4·7	66	6·4	90	10·2	105	16·9	270	8·2	287	206	$Fe_3(PO_4)_2$ and $Fe_2(SO_4)_3$						Phosphorus supplied as sparingly soluble Fe, Mg or Ca phosphates.
,, ,, (iii)	0·85	3·8	16·9	338	2·4	29			9·9	386	4·7	66	6·4	90	13·5	139	16·9	270	8·2	287	252	$FePO_4$ and $Fe_2(SO_4)_3$						Solutions relatively high in iron and in phosphorus.
,, ,, (iv)		7·5 approx.	17·8	356	4	48			10·9 including 3 as KOH	425			10·5	147	13·6	140	5·6	90			45	$Fe_2(SO_4)_3$						
Hoagland and Snyder (1933)			10	200	4	48			6	234			15	210	3	31	4	64	about 1 from salts in A–Z solutions		as required	Ferric tartrate	0·1	0·014	0·01	0·1	0·016	A balanced nutrient with an extensive micronutrient supplement in the notable A–Z solution. Cf. text, pp. 108-109.
Trelease and Trelease (1933)		5·1	7·9	158	5·3	63			9·4	358	1·2	16	6·7	94	7·9	81	6·4	102	7·9	280								The uses of ammonia and nitrate nitrogen to obtain constant pH values. Cf. text, p. 101.
Arnon (1938)			8	160	4	48			6	234	1	14	14	196	3	31	4	64			0·6 p.p.m. Fe 3 × weekly	$FeSO_4$ plus tartaric acid	0·5	0·02	0·05	0·5	0·01	A highly efficient solution incorporating ammonia nitrogen and phosphorus as $NH_4H_2PO_4$ and micronutrient additions in segregated groups of known and possible required elements.
Arnon and Hoagland (1940) (p. 477)	0·65–0·67 approx.	4·5–6·0	6	120	4	48			10	390	2	28	16	224	6	62	4	64			0·6 2 × weekly	Ferric tartrate	0·5	as required	as required	0·5	as required	Based on proportions of macronutrients absorbed by tomato. An efficient solution for sand and water cultures, especially for high light intensities.
Long Ashton Solution (as given in Part II)	0·5 approx.	5·5–6·0	6·7–15	134–300	3	36	1·3	30	3·3–5	130–295			10–20	140–284	4	41	3	48			5·6	Ferric citrate	0·55	0·064	0·065	0·37	0·02	An efficient solution containing sodium for use in sand cultures with a wide range of crops.

To face page 96]

TABLE 19B

Other Typical Nutrient Solutions Classified According to Macronutrient Salt Composition

Left-hand columns: concentration as milligram equivalents per litre—m.eq./L.
Right-hand columns: concentration as parts per million—p.p.m.
Only one column: concentration as parts per million—p.p.m.
(Approximated data)

Reference	O.P. (ats.)	pH	Ca		Mg		Na		K		N as NH_4	N as NO_3		P as PO_4		S as SO_4		Cl		Fe	Source of Fe	Mn	Cu	Zn	B	Mo	Remarks
(i) 3-*Salt Types*.																											
Hibbard and Gershberg (1924)	·25 approx.		1	21	3	36			1·8	70		1	14	5·4	56	3	48										Used for seedling growth of wheat.
Eaton (1931)			12	240	6	72			3	117		12	168	9	93	6	96			·8	ferric tartrate	0·5			1		Large-scale irrigated sand beds.
Wynd (1933)			9·8	196	24·6	296			2·5	98		9·8	137	7·5	77	24·6	394										Suitable nutrient for growth of orchids.
Weinberger and Cullinan (1936)			12	240	4	48			3	117		12	168	9	93	4	64			as required	ferric citrate	0·5			0·5		Used for growth of one year old peach trees in sand culture.
Blake *et al.* (1937)		4·2	9	180	4·4	53			1·5	58		9	126	4·4	45	4·4	70			as required	$FeSO_4$	1			1		Used for growth of apple trees in sand culture.
Van der Veen (1940)	1·0 approx.	4·6	10	200	35	420			11	429		10	142	33	344	35	560			0·9	Humate iron				1·75		Used for coffee in water culture.
Chandler (1941)	0·6	4·8	10	200	4	49			2	78		10	142	6	62	4	65			1	ferric tartrate	1	0·09	0·22	0–·5		Boron nutrition of *Brassica* crops in sand culture.
(ii) 4-*Salt Types*.																											
Sommer (1926)			5·8	116	3·1	38			4·6	179		6·6	93	3·3	34	5·8	92			trace	$FeSO_4$						Experiments on Al and Si requirements, Al at 0, ·5, 1, 2, 4, 6, 8 p.p.m.
Tiedjens and Blake (1932)		various	5·8	116	4·8	58			6·3	249		9·9	139	19	196	4·8	75	5·8	206	as required	$FeSO_4$						Comparative effects of NO_3' and NH_4^+ on apple trees grown in sand culture.
Davidson and Shive (1934)	0·9	4–8	6·4	127	16·6	203			6·3	249		8·8	125	19	196	14·2	227			Flushed with 0·1% $FeSO_4$		0·5			0·5		Continuous flow cultures used for study of pH or uptake of No_3 or NH_4 nitrogen.
Batjer and Degman (1940)			9	180	4	48	12	276	3	117		12	168	9	93	4	64	9	320	as required	ferric citrate	0·5			0·5		Boron nutrition of apple trees in sand culture.
Lehr (1940)			1·8	36	0·7	8			1·6	62		1·6	22	2·9	30	1	16			14	$Fe_2(PO_4)_3$	0·5	0·05		variable		Used for a study of boron nutrition. ·042 mg/L KI, and KF and ·083 mg/L $Al_2(PO_4)_3$ included.
Hayward and Long (1941 b)	0·75 (optimum)	6·5	2	40	2·4	29			2·2	86		4	56	0·6	6	2·4	38	up to 10 meq. (355 p.p.m.)		0·3% in sand	Magnetite						Basal nutrient composition for growth of peach trees.
Lewis and Powers (1941)			14	280	10	120			8	312		14	196	9·5	98	10	160			2	either as tartrate or humate iron	0·5	0·02	0·05	0·5	0·05	Experiments on iodide toxicity. Ferric humate did not prevent chlorosis, tartrate was effective.
Alben, Hammar and Sitton (1942)		5·5–6·0	8	160	4	48	4	92	3·2	125		15	213	0·6	6	8	128					Iron and micronutrients supplied.					Deficiency experiments with pear.
Liebig *et al.* (1942)			6·3	125	3·8	45	0·2	5	2·8	109		10	142	0·3	3	2·5	40	0·2	7	0·2	$FeSO_4$	2 0·1	0·1 0·01	2 0·1	2 0·1	0·05	Relation of aluminium to copper toxicity in *Citrus* in water culture. Micronutrients reduced in later work by Liebig *et al.* (1943).
Shive and Robbins (1942)	0·5		3	60	4·4	53	4	92	3	117		4	56	4·5	46	4·4	70	3	107	as required	$FeSO_4$	0·15		0·15	0·1 (approx.)		Successfully used for a wide range of crops for sand and water cultures.
Hayward and Long (1943)			14·4 (1·4	288 28)	14·4	168	(1·5	34)	16·2 (·05	632 2)		28·8	407	5·4	56	14·4 (·75	230 12)	·45	16	5	ferric citrate	0·4	0·01	0·1	1	0·1	A high salt type of nutrient used for experiments on salt tolerance by tomato. (Figures in brackets from tap water.)
			15·8	316	14·4	168	1·5	34	16·25	634		28·8	407	5·4	56	15·15	242	·45	16								
Sideris *et al.* (1943)		4·8	2	40	2	24			1·5	59		2	28	1·5	15	34·3	867			5·6	$FeSO_4$	0·5	0·1	0·1	0·5		Used for growth of pineapple in water culture, low N level adjusted to slow growth of the plant.
Guest and Chapman (1944)		2–7	8·33	167	5	61	0·28	6	1·3	52		8·33	117	1	10	6	96	0·28	10	0·5% as Magnetite or 0·5 p.p.m. as $FeSO_4$		0·5	0·5		0·25		Experiments on effects of pH on growth of Citrus in sand cultures
Robbins (1946)			10	200	4	48			5	195		14	196	3	31	4	64			0·5		0·25	0·02	0·25	0·25	0·01	A satisfactory general nutrient for sand culture work.
Tanada (1946)		4·5–6·5	2·4	48	2·4	29			1·45	57		3·6	51	0·75	7·5	2·4	44			20	ferric tartrate	0·7	0·1	0·3	0·2		Used for coffee in aerated water cultures.
(iii) *Ammonium Nitrogen Types*.																											
Waltman (1931)			4·2	84	1·1	14			1·3	50	10	141	10	141	2·6	26	1·1	18	4·6	163	1	ferric citrate or tartrate or leaf painting with $FeCl_3$						Response of strawberry to source of nitrogen and pH.
Clark and Shive (1932)	1·0		8·8	176	14·2	170			6·3	249	2·8	39	8·8	121	19	196	17	272			0·5	$FeSO_4$	0·5			0·5		Experiments on effect of aeration on growth of tomato. Continuous flow water culture.
Tiedjens and Blake (1932)		various	5·8	116	4·8	58			6·3	249	11·2	157			19	196	16·9	270	5·8	206	as required	$FeSO_4$						Comparative effects of NH_4 or NO_3 on apple trees in sand culture.
Spencer and Shive (1933)	Best Worst	4·1 4·1	8·8 20	176 400	4·8 4·8	57 57	2·1 2·1	81 81			14 2·8	198 39	8·8 20	124 284	6·3 6·3	65 65	7·6 7·6	120 120			as required as required	$FeSO_4$ $FeSO_4$	0·5 0·5			0·5 0·5		Modified Tottingham solutions for best and worst growth of *Rhododendron* in sand culture with continuous solution renewals.
Davidson and Shive (1934)	0·9	6·8	6·3	126	14·2	173			6·3	249	8·8	120			19	196	20·6	330					0·5			0·5		Experiments on effect of pH on uptake of NH_4 or NO_3N. NH_4 given as NH_4OH, constant renewal of solution.
Trelease and Trelease (1935)		5·1 approx.	2	40	2	24			2	78	1	14	3	42	6	62	2	32			0·02	$FeSO_4$ plus potassium citrate						An efficient stable pH nutrient for use in sand culture.
Nightingale (1934)		6·0	9	180	9	108			4·5	176	9	128			13·5	140	18	288	9	320								Comparative effects of NH_4 and NO_3 on apple trees in solution and sand culture with constant renewal.
Eaton (1936)			8	160	4	48			3·2	125	4	56	11	156	0·6	6	8	128			1% Magnetite in sand		0·2		0·1	1	Low phosphate level to avoid immobilisation of iron supply.
Addoms (1937)			5·8	117	2·8	33			6·3	249	11	156			18·9	198	12·5	201	5·7	204	0·7	$FeCl_3$	0·4			0·2		A satisfactory solution for *Pinus* seedlings in sand culture.
Arnon (1937)		4–6·7	7	140	6	72			3	117	4	56			12	124	16	256			0·6	ferric tartrate	as impurities					Relation of aeration, copper and manganese to source of nitrogen for growth of barley in water culture.
Mitchell (1939)			12·2	244	14·5	176			8·2	319	10·6	150	10·6	150	24·5	253	14·5	232	12·2	433	3·4	ferric citrate						Satisfactory for seedlings of *Pinus strobus* after initial stage.
Arnon (1940)			4	80	2	24			7	273	2	28	11	154	6	62	1	16			No data		0·5	0·02	0·05	0·5	0·01	Nutrient included $Mg(NO_3)_2$ and $(NH_4)_2HPO_4$. Micronutrient supplement designated as A_5.
De Haan and Schoorel (1940)		5·6	6	120	2	24			6	234	3	42	9	128	18	186	2	32			3·4	Humate iron	0·8	0·2		0·7		Growth of tea in sand culture.
Hill and Johnston (1940)			2·8	57	absent				0·9	34	2·2	31	3·5	49	0·9	27	0·9	14	1·2	41	3	$FeCl_3$	0·19			1		Used for magnesium deficiency in apple trees.
Wagner (1940)			13·5	270	4	48			9·7	379	2·5	35	7·4	104	13·5	139	6·4	102	4·8	170	42	$Fe(NH_4)_2(SO_4)_2$	0·13	0·14		0·1		Experiments on Si deficiency.
Waltman (1940)			8·8	176	4·75	57			2·2	86	8	112	8	112	3·3	68	4·75	76	8·9	318	1		5			3		Used for growing apple and peach trees in sand culture.
Shive and Robbins (1942)	0·5		9	180	4·6	55			2·3	91	1·4	20	9	128	6·9	71	6	96			as required	$FeSO_4$	0·15		0·15	0·1		A generally satisfactory solution for sand and water culturess.
Gregory (1943, unpublished)			0·9	18	1	12			2·1	83	6·0	84	4·6	66	2·2	22					1·25 later raised to 6·25	Numerous compounds	0·5	0·05	0·02	0·5	0·02	Experiments on micronutrient deficiencies and control of iron deficiency in flax grown in water culture.
Lineberry and Burkhart (1943)		5·6	8	160	4	48			2	78	1	14	9	128	6	62	4	64			0·25		0·25	0·25	0·25	0·5		Used to grow strawberries in sand culture.
Sideris *et al.* (1943)		5·6	2	40	2	24			1·5	59	2	28			1·5	15	6·3	99	2	70	5·6	$FeSO_4$	0·5	0·1	0·1	0·5		Used for growth of pineapple in water culture. Low N level considered suitable to such slow-growing plants (also included 0·1 p.p.m. I).
Davidson (1944)			9	180	4·7	56			3·6	141	0·66	9	9	126	10·8	111	5·4	86			0·1-10	according to need			0·05	0·3		Solution used with peach trees in sand culture.
Hobbs (1944)			10	200	5	60			2·5	98	3	42	10	140	7·5	78	8	128			2	ferric tartrate	1	0·1	0·5	0·5		A satisfactory nutrient for white pine in sand culture.
Asana (1945)			1	19	2	24			0·15	6	1	14			0·45	4·5	4	64			8	$FePO_4$						Experiments on nutrition of rice in water culture.
Houghland (1947) (B type)		4·5	9	180	4·6	55			2·3	91	0·7	10	9	128	0·15	1·5	5·3	85	2·3	83	1	ferric citrate	0·065			0·09		Study of phosphate levels for potato in water culture.
Mulder (1948 a)			2·9	58	2	24			6·4	252	2·3	32	3·5	49	6·7	70	4·9	78			5	$FeCl_3$	0·25	0·07	0·06	0·04	0·008	Used in a study of molybdenum deficiency in tomato and others. Also included 1·25 mg/L $Na_2CO_3 10H_2O$.
(iv) *Miscellaneous* (*usually more than 4 major salts*).																											
Gile and Carrero (1916)		" acid "	0·4	8	0·5	6	2·9	67	1·0	62		3·5	50	1·6	16	0·45	7	0·8	29	2 or 8	as citrate or tartrate or chloride or sulphate						Growth of rice in water culture without chlorosis.
Reed and Haas (1924 a)	1·2		11·8	237	6·8	81	0·5	11	7·1	278		11·6	164	3·3	34	4·5	72	0·4	15	trace		trace					Optimum nutrient concentration for orange trees.
Wallace (1924)		6·4–6·6	1·1	23	0·8	10	7·6	175	3·1	121		7·9	110	1·7	17	1·9	30	1·7	61	14	$FeCl_3$						Used for growth of apple trees and soft fruits in sand culture.
Sommer (1928)			1	20	4	48	0·15	4	8	312		8	112	3·3	34	5	80	0·15	5	as required	$FeSO_4$	1·5	0·125	0·5	0·5		Used in study of zinc deficiency; also included 0·5 p p.m. Al, 0·25 p p.m. I, 0·25 p.p.m. F, excess SiO_2.
Davis (1930)			4	81	2·5	30	0·9	20	7·3	286		7·7	108	1·65	17	4·8	75	1·5	54	14	$FeCl_3$						Growth of apple trees in sand culture.
Wallace (1930)			3	60	2·5	30			9·9	385		8	112	1·65	17	5·5	88	1·35	47	14	$FeCl_3$						Growth of apple trees in sand culture.
Read (private communication)			4·1	82	2·5	30	0·8	18	5·6	201		6·7	94	1·7	17	4·8	73	0·8	32	14	$FeCl_3$	0·08	0·08	0·06	0·05		Reduced K level to avoid Mg deficiency in higher light intensity.
Haas (1932 a)			33·2	663	1·1	13	0·1	2	1·2	46		33·2	471	0·25	8	1·1	18	0·1	3	trace				5	trace		Satisfactory for growth of Citrus cuttings in sand culture. High calcium, low potassium and magnesium content.
McHargue and Calfee (1932 b)			7	140	1·2	14			3·2	125		8·4	117	3·1	32	1·2	19	0·75	27	3	ferric citrate						Used for growth of *Lemna* in water culture in absence of manganese.
Colby (1933)			7·5	150	3	37			3·8	150		11·1	158	0·3	3	2·9	46			as required	ferric chloride or tartrate	Hoagland's A–Z Supplement					Used for growth of prune trees in water culture.
Arnon (1937)			7	140	6	72			3	117		4	56	12	124	8	108			0·6	ferric tartrate 3 × weekly	as impurities					Used for growth of barley in water culture in work on relation of aeration, copper and manganese to source of nitrogen.
Chapman *et al.* (1937) Low N.			3·5	70	1·4	17			2·3	90		2	28	0·3	3	5·0	80			0·2	$FeSO_4$	0·55	0·06		0·5	0·009	Used for zinc deficiency in Citrus in water culture. Contained 50 other microelement supplement.
,, ,, ,, High N.			12·5	250	7·5	90			5·2	203		20	84	0·3	3	5·0	80			0·2	$FeSO_4$	0·55	0·06		0·5	0·009	Ditto
Haas (1937)			8	159	4·5	54	0·3	7	4·5	185		11·5	162	3·2	33	4·5	72	0·3	10	0·1		6·2			0·5		Used for growth of Citrus in water culture.
Arnon and Johnson (1942)		3·9	4	80	2	24	Constant, and determined by amount of NaOH used		13	507		12	170	0·18	1·8	5	80			2	humate iron and manganese	2			0·5		Nutrient composition remains in solution between pH 3 and 9, and necessitates low P level, restored daily.
Chapman and Parker (1942)			5·7	114	2·8	34	1·3	20	1	39		1 (varied between 0·2 and 1·6)	14	0·2	2	9	104	0·6	21	trace	$FeSO_4$ daily	0·5			0·5		Citrus in water culture (0·5 p.p.m. Al included).
Piper (1942)			5·8	116	4	48	1·7	39	13·6	530		9·9	139	11·2	116	9·8	157	1·7	60	2	ferric citrate	0·5	various	0·2	0·1	0·1	Experiments on copper deficiency in water culture.
Colwell (1943)			14	280	14	170	7	161	9	351		21	298	21	217	14	224			1·2	ferric citrate	0·25	0·04	0·25	0·1		Used in application of sunflower method of boron assay. (Iron given 3 × weekly).
Gauch and Magistad (1943)	0·5	5·7	5·9	118	2·7	32	2·1	48	3·25	127		7	98	2·3	24	4·3	69	1·8	64	0·5	ferric citrate	0·5			0·5		Basal solution for study of salinity effects on crops in sand culture, contained 2·1 meq. HCO_3'.
Gregory (1943, unpublished) A			0·9	18	1	12	22·1	278	2·1	83		10·6	150	2·1	22	3·1	50	0·9	32	1·25 increased to 6·25	various forms including Fe salts of SO_4, Cl, $(CN)_6$ etc.	0·5	0·02	0·05	0·5	0·02	Experiments on micronutrient deficiencies of flax and control of iron deficiency in water culture.
,, ,, ,, C			23	460	1	12			2·1	83		10·6	150	2·1	22	3·1	50	11·7	415			0·5	0·02	0·05	0·5	0·02	
Hayward and Spur (1944)	0·4–0·5	5·5-6·5	3·9	78	2·7	32	1·5	34	3·3	129		5	71	2·3	24	4·3	69	1·45	50	1		0·5			0·5		Basal solution for study of tolerance of flax to salinity in irrigated sand cultures.
Gauch and Wadleigh (1945)	0·5	5·7	5·9	118	2·7	33	2·1	47	4·1	158		7 (2) 9	98 (28) 126	4·5	47	4·3	69	1·8	64	0·5		0·5			0·5		Experiments on salinity. Extra 2 meq. HNO_3 used to adjust pH to 5·7 owing to use of tap water.
Richards (1945)		5·5	7·6	152	0·6	7			2·3	90		7·2	100	1·4	14	2	32			1·25	ferric citrate	0·5	0·05	0·02	0·5	0·05	Experiment to test methods of reducing chlorosis in water cultures and included 0·05M K_2CO_3.
Hayward *et al.* (1946)	0·34		2	40	2·4	29	0·4	9	2·6	102		4	56	0·6	6	2·4	38	0·4	14		0·3% Magnetite in sand	0·4	0·01	0·1	0·5	0·1	Salt tolerance of peach trees in large-scale sand cultures. (Figures in brackets from tap water.)
			(1·79	36)	(0·46	5·5)	(1·4	32)	(0·08	3·1)		(0·3	0·4)			(0·54	8·4	(0·4	14)								
			3·8	76	2·9	35	1·8	41	2·7	105		4	56	0·6	6	2·9	46	0·8	28								
Curtis (1949 a)			6	120	2	24	0·1	2	2	78		7	98	1	10	0·2	3	0·1	4	0·1	$FeSO_4$ 3 × weekly	0·5	0·01	0·5	0·5		0·2 p.p.m Al. Solution used for avocado and Citrus in experiments on oxygen requirements.

Other Sources of Nitrogen in Culture Solutions

A great many nitrogenous compounds have been tested at various times as possible sources of nitrogen and in some instances certain species have been able to tolerate them or even to thrive in their presence. One of the first to investigate these possibilities was Hampe (1868), who compared the effects on maize in water cultures of using ammonium phosphate with a number of organic nitrogen compounds. The best growth was obtained with the ammonium phosphate closely followed by potassium ureate. This was greatly superior to glycine and hippuric acid was the least suitable. Classical experiments of considerable interest were made by Hutchinson and Miller (1912), who used special care to maintain sterile conditions in water cultures. The experimental plant was the pea, and nitrogen was always supplied at 80 p.p.m. The compounds tested gave widely different results and were grouped into three classes in order of the degree of their assimilation as follows :—

Good ...	...	Barbituric acid, urea, acetamide, ammonium N, formamide, alloxan.
Fair ...	...	Glycine, alanine, guanidine hydrochloride, cyanuric acid, oxamide, peptone.
Bad or Nil	...	Hippuric acid, trimethylamine, hexamethylene tetramine, hydroxylamine hydrochloride, methyl carbamate.

Tetranitromethane was highly toxic. Hutchinson and Miller (1912) also gave an extensive and valuable review of the work up to that time on the use of numerous organic and inorganic sources of nitrogen such as nitrite or hydroxylamine, and especially of work using sterile cultures. The results reviewed there showed that the whole subject was highly controversial and full of contradictory results. Nitrite in high concentrations was apparently toxic, but at 20 p.p.m. pea plants were reported to achieve complete assimilation, and low concentrations were generally equal in value to nitrate for many plants. Hydroxylamine, on the other hand, was apparently unavailable to maize and barley, as also were hydrazine and amidosulphonic acid. Some amines and amides were found to be assimilable. Amino acids such as aspartic and glutamic acid and their amides were also among those considered as potential sources of nitrogen.

Some of the more recent work may also be considered. Mevius and Dikussar (1930) studied the effect of nitrite on maize. They found that at neutral or alkaline pH values, nitrite was not toxic and no injury was observed even at 200 p.p.m. N. At lower pH values injury occurred to the roots and the maximum concentration that could be tolerated rapidly fell. This type of result was confirmed recently by Curtis (1949 b) for citrus and avocado with nitrite concentrations between 1 and 20 p.p.m. The work of earlier experimenters, including Mazé, and Birner and Lucanus, was also reviewed and the occurrence of root injury was noted in each instance due to unsuitable pH values. Fraps and Sterges (1935) also found that nitrite was less injurious to oats, maize and cotton at pH 6-7 than at pH 3-4 when grown in continuously renewed water cultures. They considered, however, that nitrite was injurious to citrus in water culture even in the presence of 700 p.p.m. NO_3'. No work has been published on the relation of micronutrients to the utilisation of nitrite by plants. Work on molybdenum in this connection should be of especial interest. Unpublished work of the writer indicated that, whereas tomatoes in sand culture tolerate 6 m.eq.NO_3' (84 p.p.m. N), but do not respond greatly to increases of molybdenum over the normal, cauliflower plants appear to be benefited thereby. The latter, however, tend to wilt during warm conditions, owing apparently to poor root action in the presence of nitrite. Bitcover and Wander (1950) observed that citrus trees in gravel culture wilted when given urea. They found that 42 mg. NO_2' nitrogen was produced in 3 days after giving fresh solutions, and that subsequent oxidation of the nitrite to nitrate probably caused a shortage of oxygen to the roots.

Beaumont *et al.* (1931) tested the response of tobacco in sterile cultures to different sources of nitrogen. Nitrate was assimilated well, asparagine and ammonium nitrogen were fair sources, whilst other amino acids including glycine were poorly assimilated.

Beaumont *et al.* (1933) compared the value of nitrate, ammonium and urea nitrogen at 84 p.p.m. N for grasses and clover in water cultures. Nitrate was best for grasses, urea

fair and ammonium nitrogen toxic, whilst urea was superior to the others for clovers and ammonium nitrogen was highly toxic even in the presence of solid calcium carbonate. Hewitt (1947 unpublished) also found that ammonium salts were toxic to clover, but not to rye grass, which also assimilated urea without injury. Pirschle (1931 a) found that yields of maize and rape with urea varied greatly with change in pH. They were poor at low pH and best at pH 7-8. Cauliflower at Long Ashton tolerated urea up to ·01 M. nitrogen.

Virtanen and Linkola (1946) found that pea plants can assimilate nitrogen from aspartic acid, whereas wheat and barley cannot. These plants also cannot utilise glutamic acid, but are able to assimilate glycine and alanine. Pea can use glycine, but alanine causes root abnormality. Audus and Quastel (1947) found that amino acids varied considerably in toxicity to seedling growth. Glutamine was generally non-toxic, but tryptophane was not tolerated. Wood and Hone (1948) found that oat seedlings in water culture assimilated oximinodicarboxylic acids at 10^{-3} M, but hydroxylamine was toxic even at 10^{-6} M. Molybdenum was given at 0·05 p.p.m. Recent work on the utilisation of nitrogenous compounds by plants was reviewed by Ghosh and Burris (1950), who also carried out further experiments. Sterile sand cultures were used, but the plants were only grown for short periods of 4-8 weeks from germination (cultures were tested at random for maintenance of sterile conditions). Tomato and clover behaved similarly in being able to assimilate nitrogen from a wide range of amino acids and amides, as well as from nitrate. Tobacco, on the other hand, was much less adapted to utilising these sources of nitrogen and growth was poor with most of them. Nitrate was superior to ammonium nitrogen for all plants and so also was nitrite for clover and tomato. Hydroxylamine was highly toxic to all three plants and oximes although not toxic were not assimilated. Asparagine, glutamine, glutamic and aspartic acids, glycine and histidine were among the best sources of organic nitrogen for tomato and clover and were generally comparable with nitrate and superior to ammonium nitrogen.

There is evidently considerable scope for further research on the conditions favouring the assimilation of nitrogen other than as nitrate or ammonium nitrogen. The level of micronutrients and nature of the species are probably of considerable importance. Results indicating effective assimilation need to be checked with regard to possible nitrification. Arnon (1937), however, found that little or no nitrification of ammonium compounds occurred in water cultures which were not kept sterile. Davis (1927) found that by taking precautions such as use of sterile water and sterilised sand and pots it was possible to prevent nitrification of ammonia even in large sand cultures for a considerable period. Results that showed little assimilation may need repetition in the presence of molybdenum.

The Influence and Control of pH of the Nutrient Solution

Influence of pH

It is convenient at this point to consider the importance of nutrient pH as this subject is closely related to nutrient composition and especially to source of nitrogen.

The majority of efficient nutrient solutions have pH values between 4·5 and 6 and these limits are usually associated with healthy growth of many plants as pointed out also by Arnon and Hoagland (1940), Livingstone (1942), Shive and Robbins (1942) and Robbins (1946). Injury to roots and depression of nutrient uptake, particularly calcium, may occur, especially at pH values of 4 or less according to Niklewski *et al.* (1928), Arnon and Johnson (1942), and Arnon *et al.* (1942), whereas Hewitt (1947 b) (1948 a) found that manganese uptake may be increased.

The pH limits at which injury occurs appear fairly well defined at somewhere about 3-3·5 for severe injury and 3·5-4 for marked depression of growth at the lower end of the scale, and at about 9 and 8·5 respectively at the upper end, as shown by the work of Hoagland (1917) for barley in water culture, Alstine (1920) for buckwheat in water culture, Salter and McIlvaine (1920) for wheat, maize, soya bean and buckwheat in water cultures, Morris and Crist (1927) and Waltman (1931) for strawberry in sand culture, Arnon (1937) for barley, Arnon and Johnson (1942) for lettuce, tomato and Bermuda grass in water culture, Guest

and Chapman (1944) for citrus in sand and water culture where the limits may extend to pH 4 and 9 for healthy growth. Salter and McIlvaine (1920) found that germination of many crop seeds was unaffected at pH values between 4 and 7-8, but was markedly reduced at pH 3·0 and 2·3, and varied considerably from one crop to another. McCall and Haag (1921) however, using sand cultures which were small and in which buffering was probably ineffective, found that wheat made best growth at pH values below 4 and that iron deficiency supervened at all higher values. Schappelle (1942) considered that pineapple grew best in water culture at pH 4 and still made fair growth at pH 3. The plants were killed at pH 2·5. pH levels were checked daily in this work and the solution was stirred by aeration in 22-L. jars. Rice, coffee and tea also appear to be favoured by relatively low values, about pH 4, according to s' Jacob (1939), van der Veen (1940) and Gadd (1928). Van der Veen (1940) found, however, that *C. robusta* was less tolerant of pH values around 4·0 or below than *C. arabica*, and there was a sharp optimum at pH 4·5-5·0.

At high pH values precipitation and non-utilisation of iron compounds is likely and is discussed later. Other nutrients, especially calcium and phosphorus, may also become unavailable and in some instances also manganese, according to Guest and Chapman (1944). For precise studies on the effects of pH on plant growth precautions should be taken to avoid changes in the concentration of the principal nutrient ions. The data of Tarr and Noble (1922) indicate the possible magnitude of these changes :—

Tarr and Noble (1922)

Conc. of ions	pH	3	4	5	6	6·5	7·6
Ca p.p.m. ...	...	96	95	95	94	58	10
Mg ...	...	127	130	131	132	125	90
PO_4 ...	...	263	268	268	263	247	194

Similar effects were observed by Reed and Haas (1924 b).

The precipitation of phosphate at high pH values and the corresponding immobilisation of iron was partially overcome by Edgerton (1942) by substitution of orthophosphate by metaphosphate. In this way good growth and freedom from chlorosis in apple trees in aerated water cultures could be obtained up to pH 7·5 with iron as tartrate. 0·005 M metaphosphate was an adequate supply of phosphorus.

In order to be independent of such effects as these Arnon (1937) and Arnon and Johnson (1942) prepared their basal nutrient solution, used to study the effect of pH on growth of various crops in water cultures, so that the various components were all in solution at the *highest* pH to be investigated. This was done by analysis of the soluble constituents remaining after precipitation of a part of the nutrients when the pH of a nutrient solution was raised to 9. The solution remaining contained only 0·00006 M phosphate in the experiment with pH values up to 9, or 0·004 M in solutions with a maximum pH of 6·7 ; iron and manganese were supplied as tartrates or as humates. A similar technique was employed for the study of pH on growth of citrus in sand or water culture, by Guest and Chapman (1944), with the addition of magnetite to supply iron and bentonite as a general ion-exchange adsorbent in the sand cultures. An interesting result of precipitation due to elevated pH was recorded by Haas (1932 a), who found that precipitation of calcium phosphate occurred in water cultures at pH 7. The precipitation occurred *on* the roots of citrus plants and resulted in impeded aeration and root rots developed. Waltman (1931) found that phosphate accumulated in sand at pH 7 or over.

Production of Specified pH Values

The production of a desired pH in a nutrient solution may be achieved in a number of ways. The simplest is the addition of requisite amounts of sulphuric acid or sodium hydroxide and compensation for changes in the sulphate and sodium levels by addition of sodium sulphate, Arnon and Johnson (1942), Guest and Chapman (1944). Eaton (1941 c), Hayward and Long (1941 b) and Gauch and Wadleigh (1945) used nitric acid to regulate the pH of solution

prepared with tap water in large-scale experiments and simultaneously added a significant proportion of the total nitrogen supply. The use of different phosphates is also a useful method. This device is used in the Rothamsted solutions, Brenchley (private communication) where differential variation of KH_2PO_4 and K_2HPO_4 allow a range of pH from 4·5-6·2 without change in total phosphate concentration. Robbins (1946) also noted the relative acidity obtainable by use of different phosphates as follows : KH_2PO_4 pH 4·8-5·0, $Ca(H_2PO_4)_2$ pH below 4·0. The same procedure was used by Hoagland (1917) and by Trelease and Trelease (1933). Wadleigh *et al.* (1937) varied the nutrient pH between 3 and 8 by differential concentrations of a series of compounds ranging from H_3PO_4 to K_3PO_4 and also KOH. Zinzadze (1926) used a combination of different phosphates of iron or calcium with ferrous sulphate to produce various pH values from 3·5-7·0.

pH Stability

The stability of pH values in nutrient solutions depends on the source of nitrogen, rate of solution renewal and probably other factors also. Control of these variables has been employed to obtain pH stability. It is necessary first to consider the changes in pH that occur as the result of their influence.

The effects of nitrate and ammonium nitrogen on pH changes are important for several reasons considered here. The magnitude of the changes may be great and toxic pH values readily occur especially when ammonium nitrogen is given and solution renewal is infrequent. Loo (1928) recorded pH changes from 5-6 down to 2·8-3·5 in water cultures, when various ammonium compounds were used to supply nitrogen. These lower values are likely to cause injury to many plants. Clark (1933) found that injury at low pH values might be more pronounced in the presence of ammonium nitrogen. It is difficult to maintain constant pH values in solutions composed solely of one or the other source of nitrogen.

A number of experiments have been made in which the solution was continuously renewed, in some instances at high rates up to 36 L per day, Nightingale (1934). Wadleigh *et al.*(1937) grew maize in sand culture with continuous solution renewal (flowing culture) at the rate of 1 L/day. The pH of the effluent of the nitrate solutions rose from 4·8 to 6·5, whilst that of solutions containing 2·8 me.q. NH_4^+ and 4·4 m.eq. NO_3' fell from 4·8 to 4·0. Clark and Shive (1934) found that with solution renewal at 2 L/day in water cultures used to grow tomatoes, the effluent pH had risen from 4 to 5·2 or had fallen from 7 to 6·2, when solutions containing 5·8 m.eq. NO_3' or 5·6 m.eq. NH_4^+ was used. Minimum pH change occurred at pH 6·0. Davidson and Shive (1934) found that the rate of solution renewal had to be raised to 6-8 L/day to check pH changes in the effluent from peach trees grown in sand cultures. Clark (1933) (1941) grew strawberries in sand cultures with solution renewal at 12 L/day and found that the change in pH in the effluent was only about 0·2 between units of 3·2 and 6·4. Tiedjens (1934) found that rates of 10-24 L/day controlled the pH change to within $\pm$ 0·2 unit when apple trees were grown in sand culture. A more detailed investigation of the pH changes induced by ammonium or nitrate nitrogen in sand or water cultures supplied with continuously renewed solutions was made by Nightingale (1934) and Clark (1941). Nightingale found that the pH of the solution adjacent to the roots of apple trees might differ greatly from that of the general effluent. In water cultures he found that at the high rate of renewal of 36 L/day, the pH value of an ammonium type solution (Table 19 B) was controlled to $\pm$ 0·1 unit at 6·0 when measured 2 cm. away from a root, but that the pH fell to 5·4 at the root surface. A similar (reverse) degree of change was found for a nitrate type solution which was maintained at pH 4·5, but rose to 5·2 adjacent to the root surface. In sand cultures the pH changes were even greater and at the root surface the pH of the ammonium type fell from 6·0 to 4·0 and that of the nitrate type rose from 4·5 to 5·6, although it was still 4·5 only 1 cm. away. Nightingale remarked " it is obviously impossible in sand cultures absolutely to control the pH of the solution bathing the roots, if the plant is absorbing from the nitrogen-containing salt of the solution in largest part ammonium from ammonium sulphate or nitrate from calcium nitrate." It was concluded that up to 4 times the concentration of ammonium nitrogen could be tolerated in *solution cultures if renewed*, than in *sand*

culture where the pH fell to 2·8-3·0 at the root surface in the presence of 36 m.eq. NH_4^+ due to more restricted diffusion, which caused pronounced root injury. Clark (1941) found similar effects with strawberries grown in sand culture at renewal rates of 12 L/day, but the differences between root film and general effluent were less marked. Gile and Fenstell (1943) and Solberg (1935) also found marked gradients of pH between root surfaces of wheat, maize and beans and the bulk of the sand in which they were grown.

Control of Nutrient pH

The differential effect of nitrate and ammonium nitrogen on pH changes has been adapted to the purpose of devising a nutrient solution in which the pH does not vary appreciably even when the solution is not renewed at the high rates described. This principle was applied by Zinzadze (1926), who devised several nutrient solutions having pH values between 4 and 7. Variations in pH were minimised by adjustment of the relative amounts of ammonium and nitrate nitrogen and of such salts as $Ca_3(PO_3)_2$, $Ca(H_2PO_4)_2$, $FePO_4$, $Fe_3(PO_4)_2$ and $FeSO_4$. A more elegant and accurate control of pH stability was achieved by Trelease and Trelease (1933) (1935) by careful adjustment of the NH_4/NO_3 ratio. The pH values were stabilised at the following levels by the ionic ratios shown below :—

NH_4NO_3 ratio	... 50 : 50	80 : 20	(85 : 15)	95 : 5
pH stabilised at	... 4·3	5·1		6·0

The actual initial pH values were obtained by adjustment of the KH_2PO_4/K_2HPO_4 ratio. The results were obtained for wheat grown in water culture. Clark and Shive (1934) working with tomato in aerated water cultures provided with solution renewal found that a pH of 6-6·2 was stabilised by a NH_4NO_3 (ionic) ratio of 28 : 29. It would appear from this that attempts to stabilise pH by this means must be tested for the crops concerned and may vary with the type of other nutrient salts applied. Pucher *et al.* (1947) found that the pH values of solution used to grow *Bryophyllum calycinum* reached the following limits after 70 days :—

% NH_4	0	50	75	100%
pH	5·5	5·1	4·5	4·7

The inclusion of a limited amount of ammonium nitrogen to stabilise pH values between 4·5 and 6·0 was also recommended by Arnon and Hoagland (1940), Shive and Robbins (1942) and Robbins (1946). Concentrations of 1-1·4 m.eq. NH_4^+ are suitable for a total nitrogen level of 10-15 m.eq. The supply of both NH_4^+ and all PO_4''' as the salt $NH_4H_2PO_4$ was used by Arnon (1938) ; this reduces the tendency for pH drop, as the absorption also of the phosphate ion by the plant is inevitable.

When it is necessary to maintain pH values without the use of more than one source of nitrogen, the method of continuous renewal of the culture solution is the most satisfactory. Consumption of water is, of course, greatly increased, but the nutrient solutions may often be used again after passage through the culture, provided they are tested and the depleted nutrients and hydrogen ion concentration are restored. Results reviewed above show that in order to be effective, rates of the order of 12 L/day or more are necessary, especially for sand cultures.

The volume of the culture solution was found by Barnette (1921) to affect the change of pH and availability of iron compounds. The application of relatively large volume water cultures either stirred, or agitated by aeration, was adopted by Arnon (1937), Arnon and Johnson (1942), and Arnon *et al.* (1942) as an alternative to rapid solution renewal. Frequent checking and correction were made when necessary, daily, Arnon (1937), Guest and Chapman (1942), Schappelle (1942), twice daily, Arnon and Johnson (1942), but variation was only of the order of 0·1-0·2 pH unit. An ingenious device was adopted by Arnon and Johnson (1942), and Arnon *et al.* (1942) to maintain pH values in the large tanks used in these experiments. Preliminary trials were first made to determine the daily requirement of dilute (N/100) acid or alkali to restore the pH to the required values. Half-litre conical flasks filled with acid or alkali were then inverted over a beaker. A bent capillary tube allowed the

contents to drip into a funnel leading to the bottom of the tank just above an aerating point to ensure rapid diffusion. The rate of drip from the capillary was controlled by the distance between the outlet and the liquid level in the beaker as in the well-known Shive and Stahl (1927) delivery method. This arrangement ensured constancy to within $\pm$ 0·2 pH unit except at pH 8-9 where the control was less close.

Tarr and Noble (1922) attempted to use 0·005 M potassium acid phthalate and phosphoric acid as a buffer system to maintain pH control, but this solution entailed the use of sterilised cultures, seeds and materials to avoid mould growth on the phthalate.

The influence of pH appears to be relatively unimportant between 4·5 and 7·5, provided that iron remains available and that the gradients close to the root surfaces are not widely different from those determined for the solution as a whole. Further detailed discussion of effects of pH on growth are given by Small (1946).

The Supply and Utilisation of Iron in Nutrient Solutions

The supply of iron to culture plants may be achieved in several ways. Some of these have special value in sand cultures, as for example the use of magnetite ; others appear to vary greatly in effectiveness according to the type of nutrient solution, for example ferric phosphate ; yet others are of particular value for water cultures and even more so at elevated pH values, for example humate iron.

Solid Iron Compounds

Solid, relatively insoluble, iron compounds were first used in sand cultures by Salm-Horstmar (1849) at the rate of 0·01 g. Fe_2O_3 per culture and later, Salm-Horstmar (1851), as Fe_3O_4 (actually purified from manganese) or as $FePO_4$ or $Fe(OH)_3$ which were judged to be good sources of iron in sand culture experiments when given at ·01-·025 g. per 60 g. of sand. Wolkoff (1918) used rust and Holt and Volk (1945) used finely ground iron ore as sources of iron for sand cultures. Magnetite (native Fe_3O_4) has frequently been used as a source of iron in sand cultures, especially when irrigated, as the precipitation of iron compounds in the reservoirs is eliminated. Magnetite appears to be a satisfactory and readily available source of iron for many plants with certain limitations as shown below in Table 20.

Table 20.

Use of Magnetite in Sand Cultures

Reference	Concentration	Crop and Remarks
Eaton (1936)	0·1%	Good for maize even at pH 8 in irrigated cultures.
Chapman and Liebig (1938) (1940) ...	0·1% (Finely ground)	Tomato and orange ; effectiveness determined by pH and phosphate supply.
Chapman (1939)	0·1–0·5% (particle size 1 micron.)	Citrus ; effectiveness determined by presence or absence of $CaCO_3$ and frequency of nutrient recirculation.
Eaton (1941 a)	0·5%	Satisfactory for several crops in irrigated cultures.
Hayward and Long (1941 b)	0·3%	Peach trees at pH 6·5 in irrigated cultures.
Magistad *et al.* (1943)	0·2%	Beans in irrigated cultures.
Guest (1944)	0·5–1·0%	Citrus ; satisfactory at pH 8 if finely ground.
Guest and Chapman (1944)	0·5–1·0% (finely ground)	Toxic impurities (zinc, etc.) liberated at low pH.
Hayward *et al.* (1946)	0·3% (passing 200 mesh)	Peach trees in irrigated cultures at pH 6–6·5.
Hewitt (1949 a)	0·1%	Sugar beet at pH 6.

Evidently the normal concentration lies between 0·1 and 0·5% by weight of the sand. Several workers emphasise that the magnetite must be finely ground. Guest (1944) found that the degree of fineness was an important factor in the success of this source of iron, and reduced the particle size by grinding for 120 hours in a ball mill. Some samples of magnetite may contain toxic amounts of other heavy metals that are liberated when brought into contact with solutions of low pH, around 3-4. Guest and Chapman (1944) found that magnetite yielded appreciable amounts of chromium, copper, nickel, and zinc to a culture solution at pH 3·0. Chapman (1939) found that the solubility of iron from magnetite was greatly increased by the presence of carbon dioxide dissolved in the solution. The effectiveness of magnetite was greater at lower pH values, and when nutrient flushings were less frequent, in experiments where calcium carbonate had been mixed with the sand. Eaton (1936) and Guest (1944) concluded that magnetite was adequately available at pH values up to 8. Eaton (1936) and Chapman and Liebig (1938) found that phosphate tended to immobilise iron in magnetite. At pH 6·3 tomatoes and orange trees were green when supplied with 0·1% magnetite and 4 p.p.m. of phosphorus. They were chlorotic when given 10 p.p.m. phosphorus at the same pH and green again at pH 5. Chapman and Liebig also considered that magnetite may fix phosphate and should not be used in experiments designed to study effects of phosphate levels.

Inorganic Salts

Sachs (1860) was probably the first to use ferrous sulphate, and since then this compound has been used frequently in culture solutions as shown in Tables 19 A & B. Ferrous sulphate is a readily soluble salt and provides an easily available source of iron, but in common with many inorganic iron compounds hydrolysis (and oxidation) followed by precipitation occur rapidly at pH values of 4 and upwards, and iron deficiency, especially in water cultures, may appear rapidly, as for example in the experiments of Spencer and Shive (1933) and Martin (1935). For this reason iron requirements in water cultures are often met by frequent periodical addition of iron compounds according to the appearance of the plants. Tiedjens and Blake (1932) and Davidson and Shive (1934) found that apple and peach trees, even in sand cultures with continuous nutrient supply, required frequent additions of ferrous sulphate, and they flushed the cultures daily with as much as 1 L. of 0·1% $FeSO_4$ solution in order to prevent chlorosis. Spencer and Shive (1933) found that *Rhododendron* also was unable to absorb sufficient iron from ferrous sulphate if the pH were 6·5-7 when the solution was continuously renewed at 1 L/day, unless 400 ml of a solution of ferrous sulphate containing 100 p.p.m. Fe was given twice weekly, but that as little as 0·5 p.p.m. Fe was effective at pH 4·1-4·6.

Ferrous ammonium sulphate was used by Martin (1935) for pineapple in water cultures and also for maize by Olsen (1935).

Attempts to overcome the tendency to hydrolysis and precipitation of iron compounds have also been made by the addition of an organic acid radical. Trelease and Trelease (1935) used a mixture of ferrous sulphate and potassium citrate as a means of maintaining a supply of available iron. This was first suggested by Reed and Haas (1924 c) in a short paper lacking details and was applied by Hopkins and Wann (1927) to a solution containing ferric chloride where 0·049 g. potassium acetate was included for each 5 p.p.m. iron present. Arnon (1938) used a mixture of 0·5% $FeSO_4 7H_2O$ and 0·4% tartaric acid at the rate of 0·66 ml/L 3 times weekly.

Ferric phosphate was introduced by Knop (1861) and this no doubt seemed a logical approach to the problem, since any simple inorganic ferric salt would be converted to phosphate in nutrient solutions containing that ion. The use of ferric phosphate attained considerable popularity, especially in the early water culture experiments carried out in the United States. Ferric phosphate is only very sparingly soluble and the availability of the iron is further reduced by elevated pH values, Alstine (1920). Chlorosis was a frequent feature of water cultures supplied with ferric phosphate when nitrogen was supplied only as nitrate, as in the experiments of Jones (1920), Barnette and Shive (1923), Jones and Shive (1921 a, b) (1923). Ferric phosphate was effective in the presence of ammonium sulphate, Jones and Shive

(1921 b). The ferric phosphate was generally added as a small amount of freshly prepared suspension. Tyner (1935) used solid ferric phosphate mixed at the rate of 1 g. per 2·5 kg. of sand.

The comparative freedom of sand cultures from chlorosis as compared with water cultures may be partly ascribed to the availability of ferric phosphate when retained on the sand particles, whilst in water cultures precipitation causes it to fall to the bottom of the culture vessel. To digress slightly, in connection with this point, Brenchley (private communication) found that chlorosis in water cultures was markedly reduced, once roots reached the bottom of the culture jar. On the other hand, Barnette (1921) found that chlorosis in water cultures supplied with ferric phosphate was progressively delayed as the culture vessels were increased in size from 250 ml. to 2,000 mls., mainly owing to more delayed rise in pH. Evidently when iron is precipitated in water culture, both the size and shape of the culture vessel may affect the incidence of chlorosis in opposite ways according to the degree of root development and rate at which the solution pH alters.

Duggar (1920 a, b) found that the efficiency of ferric phosphate was considerably increased by the preparation of what he termed " soluble ferric phosphate." This was prepared by admixture of 50 g. ferric citrate and 55 g. disodium phosphate in 100 ml. water and subsequent crystallisation of the mixture. Use of this preparation resulted in greatly increased yields with wheat and maize over those obtained with ordinary ferric phosphate. The formation of a new compound was not proved and there is no evidence that it was superior to the use of ferric citrate only.

Ferrous phosphate was also introduced early in the history of culture experiments by Birner and Lucanus (1866) and obtained great popularity after inclusion as a major constituent able to supply both iron and phosphorus in the well-known van der Crone's (1904) solution. Ferrous phosphate is appreciably more soluble than the ferric salt and provides a slightly higher concentration of available iron whilst its relatively low solubility ensures a large, steadily available reserve, without the disadvantages of either a toxic concentration of iron or loss of available iron due to steady precipitation of a less soluble ferric iron compound. In spite of this, ferrous phosphate has not been widely used except in solutions closely allied to Crone's, e.g. those of Mevius (1927) and Merkenschlager (1927) and also by Stoklasa *et al.* (1913) where the bulk of the phosphate was in soluble form, and by Zinzadze (1926). Crone's solution and close modifications, however, appear to be particularly favourable for small water cultures and may be especially suitable for plants highly susceptible to chlorosis in water culture, e.g. flax, as noted by Brenchley (private communication). A modified Crone's solution has been popular at Rothamsted for several years ; (Table 19 A).

Ferric chloride was used by Schimper (1890) and was included in several subsequent solutions. Ferric chloride is readily soluble, but is also readily hydrolysed and in the presence of acid phosphates, e.g. K_2HPO_4 and KH_2PO_4 produces free hydrochloric acid whilst ferric phosphate is precipitated. In spite of these disadvantages ferric chloride has been consistently used in the Rothamsted solution, Brenchley (private communication).

Gregory and Brenchley (1945 unpublished data), Brenchley and Warington (1945 unpublished data) studied the response of flax to growth in nutrient solutions in which the sparingly soluble phosphates of Crone's solution were partly or completely replaced, in multiples of one-fifth, by soluble phosphate, and the iron normally supplied as ferrous phosphate was similarly replaced by equivalent amounts of ferric chloride. Chlorosis was nil or only trace up to two-fifths' replacement, slight at three-fifths' replacement, but beyond this chlorosis was marked and yields were sharply reduced.

Ferric nitrate was used to supply iron in culture solutions devised by Hartwell and Pember (1918), McLean and Gilbert (1926) and Trelease and Trelease (1935). Gines (1930) found that rice was able to tolerate relatively high concentrations of ferric nitrate, but that ferric phosphate gave better results, although it was required at higher concentrations.

Ferric sulphate has been less frequently employed. It was used by Zinzadze (1926) in varying amounts in five solution types designed to maintain five pH ranges. Where ferric sulphate was the sole source of iron the weights used were 0·25-0·32 g/L (supplying 45-57

p.p.m. Fe), and in those types where high concentrations of ferrous or ferric phosphate were used to supply phosphate, ferric sulphate at the rate of 0·02-0·04 g/L was still included. Ferric ammonium sulphate used by Brandenburg (1933) and Arndt and Hoffman (1937) replaced the plain sulphate in solutions based on those of Zinzadze (1926) in experiments on copper deficiency, on account, probably, of its greater purity. Olsen (1935) compared ferric sulphate with ferrous sulphate and found the ferric salt superior at pH 7 for maize in water cultures, when supplied at a concentration of 12·5 p.p.m. Fe.

Some interesting comparative work was carried out by Schropp and Zoller (1934), Schropp and Soukup (1935). In these experiments (considered here together) several nutrient solutions devised by other workers including Bruch, Crone, Hoagland, Knop, Mevius, Schimper, Stoklasa and others were compared as regards their suitability for growth and ability to provide available iron for maize, rye, oat, barley, rice, buckwheat, hemp, flax, rape, tobacco, soya bean, pea, lupin, sugar beet and potato. In some experiments maize was used as a test plant in all solutions; in others all crops were compared in one or two solutions. Some comparisons were not valid owing to different seasons, growth periods or other changes, but certain comparisons can be drawn from this work. Maize was chlorotic in Bruch's (1902) solution provided with soluble phosphate and ferric chloride (Table 19) and green in Mevius' (1927) solution which was similar to Crone's but contained calcium carbonate instead of calcium sulphate. Maize was also chlorotic in Stoklasa's (1913) solution where iron was supplied as ferrous phosphate and the rest of the phosphates were present as soluble compounds, but was green in Gericke's solution in which iron was given as ferric citrate in spite of the presence of all soluble phosphates. It is thus seen that soluble phosphates require the supply of iron as citrate, whilst in the Crone's types the iron may be given as ferrous phosphate.

Complex iron cyanides have also been tested. Deuber (1926) compared the effects of potassium ferrocyanide and ferric ferrocyanide with ferric chloride, ferric citrate and ferrous sulphate on the growth of *Spirodella* and soya bean. Only low concentrations of potassium ferrocyanide were needed to prevent chlorosis, but they also markedly depressed growth. Ferric ferrocyanide was less effective in neutral solutions but at pH 5 this source of iron was considered superior to ferric citrate for soya bean when given at 5 p.p.m. Fe. Gines (1930) compared potassium ferricyanide with ferrous sulphate, ferric phosphate, ferric ammonium sulphate, ferric chloride and ferric nitrate on the growth of rice in water cultures. Though the work was not decisive, ferrous sulphate and ferric ammonium sulphate were superior to the ferricyanide. Rice was found to tolerate relatively high concentrations of the other salts. Gregory (1943 unpublished) also compared the value of potassium ferro- and ferricyanides with usual inorganic forms as sources of iron for flax in water cultures. The cyanides mainly eliminated chlorosis but depressed growth.

Organic Compounds

Organic iron compounds were introduced later than the inorganic types and the use of citrate or tartrate appears to have originated with Gile and Carrero (1916) and Hoagland (1919). These compounds are efficient sources of iron, and are relatively non-toxic. They are not precipitated by phosphate or at pH values below 7-8 and are only weakly ionised. Attempts to use ferric acetate, Marsh (1923), or ferrous oxalate, Duggar (1920 a), were unsuccessful owing to toxic effects.

The use of ferric citrate or tartrate merits more detailed discussion. Inclusion of citrate or tartrate ions to prolong the solubility of other compounds of iron has already been mentioned. The stability of ferric citrate or tartrate may be less in light. Gile and Fenstell (1943) considered that these compounds were susceptible to photo-chemical reduction and decomposition of the organic acid. The ferrous ion was then re-oxidised and precipitated, especially in aerated solutions. Rapid photo-chemical reduction of ferric citrate has been observed by the writer, especially if the ferric citrate has been prepared by fusion of ferric chloride with citric acid (see Part Two). The residual chloride or hydrogen ions appear to catalyse the reduction. Ferric citrate, however, is stable if stored in a darkened bottle. Manganese also seems to catalyse the decomposition of ferric citrate, which was found by

the writer to be more rapidly decolourised and precipitated in the presence of manganese in the light than in manganese-free solutions. Nutrient solutions prepared with ferric citrate as a source of iron remain completely soluble if stored in dark glass, but rapidly produce a precipitate, probably of ferric phosphate, when kept in plain glass bottles. The usual concentration range of iron as citrate or tartrate is from about 0·5-5·6 p.p.m. Fe, either as a part of the nutrient solution or as a frequent supplement given when judged necessary.

Olsen (1935) considered that ferric tartrate was less stable than the citrate and Parker (1927) found that even ferric tartrate reduced the availability of phosphate when the latter was given at low concentrations. Edgerton (1942) found that apple trees grown in water cultures were green at pH 4·8 and chlorotic at pH 7·5 when orthophosphate was given, even in the presence of 50 p.p.m. Fe as tartrate, but the trees were green at the higher pH when phosphate was given as metaphosphate with as little as 2·5 p.p.m. Fe as tartrate. The metaphosphate was a satisfactory source of phosphorus, but reverted in time to orthophosphate.

Tottingham and Rankin (1923) compared the effectiveness of ferric citrate with ferric phosphate, ferric sulphate and ferrous sulphate for wheat in water culture. Least chlorosis and maximum growth were obtained with the citrate, but higher concentrations of citrate—up to 50 p.p.m. Fe—were both required and tolerated than when the other salts were used (2 p.p.m. Fe). The solubility of various compounds were also compared at two pH values of 4·2 and 6·0. There was little difference for the citrate, but marked reduction for the others, especially ferrous sulphate.

French (1937) also found that high concentrations of iron, up to 100 p.p.m. as citrate, were necessary for maize in sand culture ; whereas only 25 p.p.m. Fe was required if given as ferrous sulphate, which allowed better growth, whilst the nitrate was entirely inferior ; Pearse (1937) also recorded unusually high iron requirements for apple trees grown in water cultures, especially if the roots were completely immersed in the aerated solution. Under these conditions concentrations of 100 p.p.m. Fe as citrate or chloride were inadequate, and only sufficed when the roots were supplied by a continuous spray of nutrient solution.

Gile and Carrero (1916) compared ferric citrate and tartrate with ferrous sulphate, ferric chloride and dialysed iron, each at 2 and 8 p.p.m. Fe, as sources of iron, for rice in water cultures maintained at " acid " and " alkaline " pH values. The decreasing order of effectiveness was citrate, tartrate, sulphate, chloride, dialysed iron. The superiority of citrate was particularly apparent in the alkaline solution. A similar comparison was made by Olsen (1935) with maize. Citrate was again superior, severe chlorosis occurred with ferric chloride and a trace with tartrate, whilst ferrous sulphate appeared to be toxic. Schropp (1936) prepared a series of solutions according to earlier published formulae and compared each with its original source of iron and with iron at the same concentration as ferric citrate for growth of maize. The following results were obtained :—

Schropp (1936)

Solution	Concentration of iron p.p.m.	Original source of iron	Dry wt. g. for original type	Dry wt. g. for citrate type
Aschoff	2	$FeCl_3$	·34	2·97
Bruch	3	$FeCl_3$	·36	4·08
Detmer	28	$FePO_4$	1·43	2·73
Knop	28	$FePO_4$	·40	3·54
Tollens	28	$FePO_4$	·39	4·19
Crone	83	$Fe_3(PO_4)_2$	2·68	—

Ferric citrate was obviously a superior source of iron in all comparisons.

An important innovation in the supply of iron in organic form was the use of *humate* iron proposed by Horner *et al.* (1934) and already mentioned in connection with supply of manganese and other micronutrients. The preparation of metal humates is given as follows *loc. cit.*

1 L. 30% $^{v}/_{v}$ H_2SO_4 is heated to boiling, 100-150 g. sucrose or glucose are added cautiously and simmered 3 hours ; the product is cooled and centrifuged. Concentrated KOH is added with stirring to the solid mass until alkaline to phenolphthalein (external indicator used on diluted test portions). The mixture is again centrifuged and washed with dilute KOH. The soluble potassium humate is acidified to pH 3 by dilute H_2SO_4. The humic acid which is precipitated is redissolved in 1% KOH and again precipitated. The iron (or other metal humate) is prepared by adding a neutral salt of the metal to neutral potassium humate, followed by alkali, if necessary to neutralise acid liberated by hydrolysis. Ferric humate can be precipitated at pH 3 and stored dry, although it does not readily decompose in solution. Ferrous humate is oxidised by air to the ferric state. Suitable concentrations are 2-50 mg. Fe (as humate) per ml. Metal humates are precipitated by 0·01 M Ca (i.e. 20 m.eq. Ca^{++} or above), but are soluble in the presence of high phosphate concentrations or in solutions less alkaline than pH 10. The total concentration of humate metals permissible ranges from 5-20% of the weight of humate, and ions of Cu, Zn, Mn, Cr, Ti, Al, Ni, Co, Hg, can be retained as humates in addition to Fe^{++} and Fe^{+++}.

Iron as humate was used by Arnon and Johnson (1942) for lettuce and Bermuda grass, and by Guest and Chapman (1944) for citrus. It was found especially useful in water cultures at high pH values. Lewis and Powers (1941), however, found that the use of humate iron resulted in chlorosis of maize, which was prevented by using ferric tartrate. Their solution contained dipotassium phosphate and 14 m.eq. Ca^{++} in addition to tap water. It is probable therefore that the critical [Ca^{++}] value was exceeded. s'Jacob (1939) and van der Veen (1940) used humate iron for coffee in water cultures. It was satisfactory at pH values below 5·5 or at higher pH when alternating solutions were used for iron and phosphorus supply. Humate iron at 3·45 p.p.m. was satisfactory for tea grown in water cultures at pH 5·5 by de Haan and Schoorel (1940) and de Haan (1941).

Schropp (1938) compared the effect of substituting humate iron for the normal compound used in a series of nutrient solutions. Humate iron was found to be inferior to ferric chloride in solutions prepared according to Bruch, Tollens and Knop for maize, but superior to the use of ferric phosphate in Detmer's solution. For pea, however, humate iron gave the best results in all solutions tested and similar results were obtained with soya bean, except in Crone's solution where ferrous phosphate was superior. It appears from this that maize may not readily absorb iron from humate or possibly other organic compounds as indicated by the work of Lewis and Powers (1941) and Schropp (1938) and of French (1937).

The relative yields as dry weight of plants grown in the respective solutions in Schropp's (1938) experiment are given below. Yields in Crone's solutions are given here as 100.

Schropp (1938)

Solution : a=original iron source ; b=humate

Crop	Crone		Knop		Tollens		Detmer		Bruch	
	a	b	a	b	a	b	a	b	a	b
Barley ...	100	102	88	70	68	87	87	137	65	72
Wheat ...	100	116	84	95	104	100	55	131	68	69
Maize ...	100	87	54	26	52	25	18	64	42	26
Pea	100	106	77	100	76	108	55	108	23	54
Soya bean ...	100	99	8	62	11	45	91	91	16	31

Jacobson (1951) suggested the use of ferric potassium ethylenediamine tetra-acetate (sequestrene AA) as a source of iron in water culture. A single application at 5-10 p.p.m. Fe sufficed for 6 weeks' growth of tomato, barley, maize or sunflower. pH stability was good.

Other Sources

A few other sources of iron remain to be considered. Marsh (1923) (1924 a, b) and Marsh and Shive (1925) suggested the use of ferric glycerophosphate. This compound is relatively soluble, weakly ionised, and readily available in water cultures for cereals. Marsh (1924 a, b) considered that ferric glycerophosphate was especially suited for work at high pH values and found that with its use the dry weight of soya bean plants was double that of plants grown with Duggar's (1920 a) soluble ferric phosphate in water culture experiments employing forced aeration and continuous renewal of solution. Marsh and Shive (1925) compared the effectiveness of ferric glycerophosphate, tartrate, soluble phosphate and ferrous sulphate in three solutions, namely Shive's, Tottingham's and one containing ammonium sulphate. Soluble ferric phosphate was generally superior to the others. Schropp (1934) extended this work in a test involving nine iron compounds. Soluble ferric phosphate was generally superior or equal to tartrate and glycerophosphate for maize and oat in Richter's solution based on potassium phosphate and for maize in Crone's solution, whilst ferric sulphate was best for oat in Crone's solution.

Arnon and Grossenbacher (1947) supplied ferrous iron combined with Amberlite resins for use in irrigation cultures and grew tomato plants without signs of chlorosis.

While it may be concluded that under certain circumstances one source of iron may be superior to another, it is probable that ferric citrate is generally the simplest and least injurious form in which to supply iron at concentrations between 0·5 and 5·6 p.p.m. This applies for both sand and water cultures, provided that light is excluded from the culture solution. Most plants will grow normally when supplied with iron at concentrations around 2 p.p.m. This statement may require modification if abnormal concentrations of other micronutrients, for example manganese, used by Somers and Shive (1942), Hopkins *et al.* (1944), Pearse (1944) and Twyman (1946), or zinc, copper, chromium, cadmium, cobalt, molybdenum, used by Hewitt (1948 b) (1949 a, c) are present. Certain crops, for example, rice used by Thelin and Beaumont (1934), maize grown by French (1937), flax studied by Gregory and Brenchley (1945 unpublished) and Brenchley (private communication) or *Ulex* sp. used by Millener (private communication) may have higher iron requirements or show inability to utilise many iron compounds in water cultures. Coffee may also fail to absorb iron from solutions less acid than pH 5·5 according to s' Jacob (1939).

The Micronutrients Supplied in Nutrient Solutions

Micronutrient Supplements

The inclusion of micronutrient supplements is now a generally accepted procedure, although for work in which less precision is required the use of lower grade salts may often make additions of all the known micronutrients and particularly of copper, zinc and molybdenum less necessary, as shown by Arnon and Johnson (1942), Shive and Robbins (1942), Gauch and Magistad (1943), Hayward and Spur (1944), and Houghland (1947). Prior to the work of Arnon (1938), and Arnon and Stout (1939 b), molybdenum was not purposely given except in the experiments of Hoagland and Snyder (1933) and Colby (1933). Numerous investigators however, used nutrient solutions to which additions of small amounts of several microelements were made, either as a safeguard against possible deficiency effects or to test the possibility of stimulation by as yet unrecognised micronutrients, inferred by Arnon (1938) and Hoagland (1941). Details of the more elaborate supplements are given here mainly in chronological order, but also grouped according to general composition. Early European and American work was reviewed by Schropp and Scharrer (1933).

Group I.—*B, Mn, Zn, Al and Si and halides*

Mazé (1900), (1914)	$MnCl_2 4H_2O$ 0·1 g/L. traces K_2SiO_3 and $ZnCl_2$
Mazé (1915)	$MnCl_2 4H_2O$ 0·02 g/L., $Na_2B_4O_7 10H_2O$ 0·004 g/L., K_2SiO_3 0·02 g/L., $ZnCl_2$ 0·02 g/L. (also tested Na_2HAsO_4 at 0·002 g/L.)

Haas and Reed (1927)	0·2 p.p.m. each Br, I, Al, B, Mn, Li, Ti, Sr. *Original use of term " A—Z."*
Sommer (1928)	NaCl 0·0127 g/L., B. 0·5 p.p.m., Mn 1·5 p.p.m., Cu 0·125 p.p.m., Zn 0·5 p.p.m., I 0·25 p.p.m., F 0·25 p.p.m., Al 0·5 p.p.m., SiO_2 excess.

Same composition used also by Sommer and Sorokin (1928) and by Lohnis (1937).

Brandenburg (1935)	H_3BO_3 0·5 mg/L., $MnSO_4 4H_2O$ 1-2 mg/L., ($\pm$ Cu), $ZnSO_4 7H_2O$ 0·5 mg/L., $Al_2(SO_4)_3$ 0·5 mg/L., K_2SiO_3 25-50 mg/L.
Arndt and Hoffman (1937)	H_3BO_3 0·5 mg/L., $MnSO_4 4H_2O$ 2 mg/L., $ZnSO_4 7H_2O$ 0·5 mg/L., $\pm$ $CuSO_4 5H_2O$, 2-9 μg., $Al_2(SO_4)_3$ 0·5 mg/L., K_2SiO_3 35 mg/L.
Lipman (1938)	traces B Zn Cu Al F I.
Sideris *et al.* (1938)	B Mn Cu Zn Al Br I Si Ti 0·5-1·0 p.p.m. each
Lehr (1940)	H_3BO_3 or $Na_2B_4O_7$ variable, $MnSO_4 4H_2O$ 0·25 mg/L., $CuSO_4 5H_2O$ 0·021 mg/L., $Al_2(SO_4)_3$ 0·083 mg/L., KI 0·042 mg/L., KF 0·042 mg/L.

Group II.—*Complex (" hit or miss ") supplements*

Sommer (1931)	traces of Mn, B, $\pm$ Cu, Zn, F, I, Al, SiO_2, Li, Na, Rb, Co, Ni, As, Sn, Ba, Pb, Hg.
Hoagland and Snyder (1933) (The well-known A—Z supplements)	A. 1 ml/L. of solution containing following composition as g/18L. :— $Al_2(SO_4)_3$ 1·0, KI 0·5, KBr 0·5, TiO_2 1·0, $SnCl_2 2H_2O$ 0·5, LiCl 0·5, $MnCl_2 4H_2O$ 7·0, H_3BO_3 11·0, $ZnSO_4 7H_2O$ 1·0, $CuSO_4 5H_2O$ 1·0, $NiSO_4 6H_2O$ 1·0, $Co(NO_3)_2 26H_2O$ 1·0.
	B. 1 ml/L. of solution containing following composition as g/18L. :— As_2O_3 0·1, $BaCl_2$ 0·5, $CdCl_2$ 0·1, $Bi(NO_3)_3$ 0·1, Rb_2SO_4 0·1, K_2CrO_4 0·5, KF 0·1, $PbCl_2$ 0·1, $HgCl_2$ 0·1, MoO_3 0·425, H_2SeO_4 0·1, $SrSO_4$ 0·5, VCl_3 0·1.
Schropp and Scharrer (1933)	Tested A—Z supplement on numerous crops.
Grossenbacher and Livingstone (1936)	Grouped additions based on compositions of Hoagland's A—Z solution. Weights given as mg/L. :— a. Mn ·0098 Zn ·0011 Cl ·013 b. Al. ·0008 B ·0089 Cu ·0013 c. Mn, Zn, Cl, Al, B, Cu (weights as above). d. c plus As. ·0004 Ba ·0013 Bi ·0006 Br ·0017 Cd ·0004 Co ·0011 Cr ·0007 F ·0003 Hg ·0008 I ·0019 Li ·0004 Mo ·0012 Ni ·0011 Pb ·0006 Se ·0004 Sn ·0008 Sr ·0012 Ti ·0013 V ·0004 W ·0005. e. as d but without c. f, g, h, i. as e but without Mn, B, Cu or Zn respectively.
Chapman *et al.* (1937)	mg/L. Al ·539 Sb ·012 As ·007 Ba ·686 Be ·09 Bi ·021 B ·541 Br 1·598 Cd ·011 Ce ·014 Cs ·013 Cl 7·092 Cr ·052 Co ·005 Cb ·009 Cu ·063 Er ·050 F ·019 Ga ·006 Ge ·007 Au ·019 In ·011 I ·012 Ir ·019 La ·013 Pb ·020 Li ·069 Mn ·549 Hg ·020 Mo ·009 Nd ·014 Ni ·005 Os ·019 Pd ·010 Pt ·019 Rh ·019 Ru ·010 Rb ·008 Se ·079 Si 1·4 Ag ·01 Na 4·6 Sr ·087 Ta ·018 Te ·012 Th ·023 Sn ·011 Ti ·239 W ·018 U ·023 V ·059 Y ·008 Zr ·009 ; Zn ·65 or Nil.

As a matter of interest spectrographic analysis of the compounds used to supply these elements was made. V and W showed no detectable Mo, and Al was similarly " free " of Ga.

Arnon (1938)	A_4 :—B 0·5 p.p.m. Mn 0·5 p.p.m. Cu 0·02 p.p.m. Zn 0·05 p.p.m.

Grouped additions used in detection of response to Mo	B_7 :—Mo, V, Cr, W, Co, Ni, Ti 0·01 p.p.m. each. C_{12} :—Al, As, Cd, Sr, Hg, Pb, Li, Rb, Br, I, F, Se, Be, 0·005 p.p.m. each.
Pearse (1940)	Traces B, Mn, Cu, Zn, Ni, Co, Al, Br, I, Li, Sn, Ti.
Hoagland (1941)	As Hoagland and Snyder (1933), but regrouped into A_5 (see below) type and 21 others given in A—Z, but with W added.
Barker and Broyer (1942)	B 0·5, Mn 0·5, Cu 0·02, Zn 0·05 p.p.m. each. Mo, Cl, Cr, W, Ni, Cu, Ti 0·01 p.p.m. each.
Chesters and Street (1948)	B 0·1 Mn 0·24 Cu 0·013 Zn 0·02 Co 0·01 Ni 0·01 Sn 0·016 Ti 0·018 Al 0·004 Li 0·003 Br 0·02 I 0·023 Si 0·1 p.p.m. each (Mo Nil).

Group III.—*Minimal supplements of known micronutrients*

Arnon (1940)	A_5 Supplement :—B 0·5 Mn 0·5 Cu 0·02 Zn 0·05 Mo 0·01 p.p.m. each. (Replaces A_4 given in Arnon (1938)).
Lewis and Powers (1941)	B 0·5 Mn 0·5 Cu 0·02 Zn 0·05 Mo 0·05 p.p.m. each.
Piper (1941)	H_3BO_3 0·5 mg/L. Cu 0·1 Zn 0·2 Mo 0·02 Mn 0-0·5 p.p.m. each.
Piper (1942)	B, Mn, Zn as above. Mo 0·1 p.p.m. Cu 0-1 p.p.m. (0·05 about optimal).
Gregory (1943)	B 0·5 Mn 0·5 Cu 0·02 Zn 0·05 Mo 0·02 p.p.m. each.
Hayward *et al.* (1946)	B 0·5 Mn 0·4 Cu 0·01 Zn 0·1 Mo 0·1 p.p.m. each.
Long Ashton Nutrient	B 0·37 Mn 0·55 Cu 0·064 Zn 0·065 Mo 0·019 p.p.m. each. (Mo could be advantageously raised to 0·5 p.p.m. for Brassicas.)

For highly purified solutions the following are also added : Al 0·01 p.p.m., Ga 0·005 p.p.m., Ni 0·005 p.p.m., Co 0·005 p.p.m.

Micronutrient Requirements

It may be inferred from the data given here and in Tables 19 A & B that five micronutrient elements namely, boron, manganese, copper, zinc and molybdenum are generally regarded as essential for growth of most, possibly all, higher plants, since none so far tested has failed to respond to the supply of these elements *when a sufficiently exacting technique has been used.* Responses to any other micronutrients including gallium, are still not proved, since the original claim by Steinberg (1938 b) (1941) (1943) was never confirmed in subsequent work, Steinberg (1945 a).

Although traces of some or all of the five may be accidentally included under ordinary experimental conditions, it is still true that the discovery of each has been made from the fact that occasionally (and with improved means of reagent manufacture, more frequently), plants have failed to show normal development owing to the lack of an essential micronutrient. It is therefore advisable to ensure that a small but adequate supply of each of the five is included in all nutrient solutions. The cost of the supplement is small owing to the minute amounts necessary. Furthermore, when newly identified interactions are investigated it is possible and even likely that the results may be modified by the presence or absence of the known micronutrients, and reproducible and valid results may only be obtained when the experimental plants are grown in the presence of all the known essential elements including the micronutrients at specified concentrations. A few examples will emphasise this important point. Arnon (1937) showed that the effect of aeration on the growth of barley in water culture was determined not only by the source of nitrogen as nitrate and ammonium, but also by the absence or presence of micronutrients, especially Mn and Cu. Arnon emphasised the importance of taking into consideration the microelement impurities present in assessing the relative value of nitrate and ammonium nitrogen and advised the inclusion of an A—Z

type supplement in such experiments. This attitude may be particularly relevant to the assessment of much of the earlier work on the comparative value of these sources of nitrogen. It may have been that the relatively *infrequent* instances of superiority of ammonium nitrogen type nutrients was due to accidental lack of molybdenum, since Mulder (1948 a) suggested that the need for this element is less (perhaps nil) when adequate ammonium nitrogen is given instead of nitrate. A further example is provided from experiments by Millikan (1947 b) and by the writer, Hewitt (1948 b) (1949 a, c) (and unpublished), on the ability of various metals to induce iron deficiency. It was found that the iron requirement of plants was modified by the presence of high concentrations of micronutrients and other heavy metals, and that the level of molybdenum had an important effect on the ability of these elements to induce iron deficiency. The effect produced by molybdenum was dependent also on the source of nitrogen.

The composition of the micronutrient supplement has not always been consistent in a sequence of studies. Thus in a series of experiments on the nutrition of the pineapple in water culture Sideris *et al.* (1943) supplied manganese and boron at 0·5 p.p.m., zinc, copper and iodine at 0·1 p.p.m. and no molybdenum, whereas Sideris and Young (1946) gave manganese and boron at 0·5 p.p.m., zinc at 0·6 p.p.m., molybdenum at 0·1 p.p.m., but no copper. Liebig *et al.* (1943 b) in an unsuccessful attempt to determine the need of gallium for citrus did not include either copper or molybdenum, although the nutrients, including iron salts, were subjected to purification treatments known to remove copper, and redistilled water was used. Sommer (1926) performed experiments to determine the need of aluminium and silicon for plants. Glass distilled water and " purified " salts were used and the plants were grown in waxed glass jars, but no extra micronutrients were added to any solution, with one exception where 0·008 p.p.m. manganese was included, in spite of the fact that the importance of boron and probably of zinc Mazé (1914, 1915) had already been demonstrated. A more recent example also calls for comment. Chesters and Street (1948) have re-opened the question of " auximones " and organic supplements for culture solutions. In these experiments significant stimulation of lettuce was recorded when an infusion of leaf mould was added to the purely mineral culture solution. The pH of this solution was 6·4-6·6 and the possibility that more readily available humate compounds of iron and manganese were formed from the leaf mould was not properly excluded. Also as no molybdenum was included in the nutrient solution it was possible that the " infusion " introduced this element (known to be beneficial to lettuce from work by Warington (1946)), particularly as organic plant residues are likely to be rich sources of molybdenum.

Threshold and Toxicity Levels for Micronutrients

The micronutrient elements may be toxic at even relatively low concentrations compared with the macronutrients and the useful range of concentrations is consequently narrow. For the majority of plants the following ranges may be regarded as adequate :—

	Usual Range	Suggested level for first trial
B	0·1 -1·0 p.p.m.	0·5 p.p.m.
Mn	0·1 -1·0 ,,	0·5 ,,
Cu	0·01-0·1 ,,	0·02 ,,
Zn	0·02-0·2 ,,	0·05 ,,
Mo	0·01-0·1 ,,	0·02 ,,

There are, however, numerous instances where the optimum concentrations were outside these limits. Ferguson and Wright (1940) found that turnips in sand culture required 1-1·5 p.p.m. boron. Lehr (1940) found that maize grown in water culture required ·05 p.p.m. whilst tomato and mustard needed 0·1 p.p.m. boron for maximum growth. Lehr also reviewed the work of several investigators and showed that the optimum boron requirement varied from 0·025-0·1 p.p.m. for soya bean (Minarik and Shive 1939) to 0·7 p.p.m. for lettuce (McHargue and Calfee 1933). Moinat (1943) found that boron deficiency in lettuce was prevented by as

little as 0·005 p.p.m. van Schreven (1939) concluded that the threshold for potatoes was between 0·05 and 0·07 p.p.m. Marsh (1942) showed that the boron requirements of cereals are generally less than those of dicotyledonous plants and Warington (1923) concluded that they may be well below 0·01 p.p.m., whereas for pea the lower threshold is around 0·1-0·2 p.p.m., and the toxic level determined by Brenchley (1914) between 1 and 10 p.p.m.

Reeve and Shive (1944) found that the thresholds of boron deficiency and toxicity were modified by potassium and calcium level, but tomato showed neither deficiency nor toxicity of this element when supplied with 0·5 p.p.m. B at any calcium or potassium level from 5-500 p.p.m. Toxicity effects were more noticeable at low potassium levels and occurred with as little as 1-3 p.p.m. B in sand cultures. Similar concentrations were often toxic to citrus, pecan, avocado and peach grown in sand culture by Haas (1929). Rowe (1936) found that beet tolerated up to 8 p.p.m. B in water culture and Henderson and Veal (1948) concluded that blue lupins tolerated 15 p.p.m. B in water culture and that 7·5 p.p.m. was optimum. Meyer (1946) found that 10-15 p.p.m. B was optimum for root production by the Russian latex plant, kok-saghyz. Eaton (1944) grew 50 species in sand culture with varied boron levels from 0·03 to 25 p.p.m. The distribution of best growth for 72 croppings was as follows :—

Eaton (1944)

	No. of croppings giving best growth
0·03 p.p.m. B ...	19
1	20
5	20
10	7
15	6
25	—
	72

Turnip, beet and cotton were materially benefited by 5 p.p.m. B and even 10 p.p.m. B ; most crops benefited from 1 p.p.m. B and lemon and blackberry were plainly injured at this concentration.

The threshold levels for manganese have not been widely investigated. Piper (1941) found 0·02 p.p.m. was inadequate and 0·5 p.p.m. adequate for the growth of peas. Unpublished work of the writer has shown that oat, cauliflower and clover require more than 0·02 p.p.m. for normal development and that 0·275-0·55 p.p.m. appear adequate for these and a wide range of other crops. Concentrations above 1 p.p.m. are rarely used. Arnon and Johnson (1942), however, supplied manganese at 2 p.p.m. as the humate metal (cf. Section on iron compounds) to lettuce, tomato and Bermuda grass grown in water culture. Schappelle (1942) supplied manganese at 2 p.p.m. to pineapple grown in water culture in spite of the susceptibility of this plant to induced iron deficiency. Jacobsen and Swanback (1929) considered 1 p.p.m. to be toxic to tobacco grown in water culture, and Brenchley (1910) concluded that 2 p.p.m. was the threshold of toxicity to barley in water culture. Liebig *et al.* (1942) found that manganese concentrations up to 1 p.p.m. were not toxic to citrus (Valencia orange or lemon) in solution culture. Haas (1932 b) found that 5 p.p.m. was toxic to orange, Rough lemon and Eureka lemon under similar conditions and induced chlorosis. Hewitt (1946 b) (1947 b) (1948 a) studied the responses of several crops to excess of manganese and found that many crops readily tolerated up to 5 p.p.m. in sand culture, although tolerance varied widely. Oat and sugar beet were relatively tolerant, but many brassica crops were very susceptible to manganese excess. It may be concluded that 1 p.p.m. is probably an upper safe limit and that most plants will grow well when supplied with 0·25-0·5 p.p.m. manganese.

Copper is probably the most toxic of the essential micronutrients and the normal concentrations required are much lower than for boron and manganese. Piper (1942) made a detailed study of the copper requirements of several plants grown in water culture. Most plants tolerated 0·1 p.p.m. and grew normally, but this concentration was probably excessive for tomato. Peas produced normal growth at concentrations between 0·006 and 1 p.p.m.,

and Algerian oat behaved similarly between 0·02 and 2 p.p.m. Cereals are notably susceptible to copper deficiency in the field. Brandenburg (1933) found that oats required up to 0·125 p.p.m. in water culture and grew less vigorously at 0·05 p.p.m., whilst Arndt and Hoffman (1937) regarded 0·01 p.p.m. as the lower threshold for deficiency. Brenchley (1910) determined 0·1 p.p.m. as the threshold for copper toxicity to barley in water culture. Liebig *et al.* (1942) grew citrus in water culture with 0·1 p.p.m. and found that this concentration was toxic unless aluminium was also given at 0·1-2·5 p.p.m. Guest and Chapman (1944) also found that 0·11 p.p.m. copper was toxic to citrus (sweet orange), although Haas (1949) grew Valencia orange, also in water culture, with 0·1 p.p.m. in solution. The copper requirement of some plants may be somewhat greater than indicated so far. Hoagland (1941) found that Myrobalan plum showed severe deficiency effects at 0·01 p.p.m. Cu, 0·05 p.p.m. being insufficient and 0·1 p.p.m. regarded as threshold. Sideris *et al.* (1943) grew pineapple in water culture with 0·1 p.p.m. Cu, and Schappelle (1942) used a concentration of 2 p.p.m. under similar conditions and this was found to prevent the development of root fungi. Lineberry and Burkhart (1943) grew strawberries in sand culture and supplied copper at 0·25 p.p.m. It would appear that 0·02-0·05 p.p.m. Cu is the most likely range for satisfactory results.

Tolerance to and requirements for zinc are rather greater than for copper, as may be inferred from Tables 19 A & B, and zinc is often included at concentrations equal to or even greater than manganese, as shown here in Table 21.

TABLE 21

Levels of Zinc and Manganese (p.p.m.) in Some Culture Solutions

Reference	Mn	Zn	Crop	Method
Haas (1932 a)	trace	5	Citrus	Water culture
Schappelle (1942)	2	2	Pineapple	Water culture
Liebig *et al.* (1942)	2	2	Citrus	Water culture
Liebig *et al.* (1943 b)	0·1	0·1	Citrus	Water culture
Lineberry and Burkhart (1943) ...	0·25	0·25	Strawberry	Sand culture
Reeve and Shive (1944)	0·25	0·5	Tomato	Sand culture
Guest and Chapman (1944)	0·5	0·5	Citrus	Sand and Water culture
Sideris and Young (1946)	0·5	0·6	Pineapple	Water culture
Haas (1949)	0·1	0·25	Citrus	Sand culture

Eaton (1941 b) supplied zinc to tomato and maize at 0·2 p.p.m. in water culture. Sideris *et al.* (1943) gave 0·1 p.p.m. to pineapple in water culture. Piper (1941) (1942) gave 0·2 p.p.m. to cereals, peas and legumes in water cultures. These levels are all materially greater than those commonly chosen for copper or recommended by Arnon (1938) (1940). Guest and Chapman (1944) found 2-4 p.p.m. zinc toxic to citrus, but Haas (1932 a) reported that although slightly toxic to young citrus cuttings, 5 p.p.m. benefited larger ones, but in later work, Haas (1949), the level was much reduced. Chlorosis symptoms induced in pineapple in the experiments of Schappelle (1942) were probably due to the additive effects of 2 p.p.m. each of manganese, copper and zinc. It may be concluded that many plants will readily tolerate 0·1 p.p.m. zinc in water culture and probably 0·25 p.p.m. in sand culture, but Brenchley (1914) regarded approximately 0·08 and 0·16 p.p.m. as thresholds for toxicity to barley and pea respectively grown in water culture. Early (1943) found that 0·3-1·6 p.p.m. zinc were the limits of tolerance for different varieties of soya bean in water culture.

Molybdenum is of especial interest as the toxicity/deficiency ratio is probably greater than for any other known micronutrient. No published data are available yet as to the

lower limits of molybdenum requirements. Hewitt (1950, unpublished) found that at 0·006 μg/L, i.e. 0·000006 p.p.m. (determined by tests with *A. niger*) acute deficiency effects prevail in most higher plants. Dr. S. C. Agarwala (unpublished work at Long Ashton) showed that growth of cauliflowers in sand cultures was definitely impaired with less than 0·005 p.p.m. Mo. Vanselow and Datta (1949) observed recovery of molybdenum-deficient citrus plants given 0·0001 p.p.m. Mo, and normal growth occurred with 0·001 p.p.m. Warington (1937) (1946), Arnon and Stout (1939 b), and Hewitt and Jones (unpublished) have confirmed that appreciable toxicity does not appear in many plants until more than 5 and probably 10 p.p.m. molybdenum is given. In fact, Agarwala (unpublished) and Hewitt and Jones (unpublished) found that 5 p.p.m. may produce better growth in cauliflower, as judged visually and by dry weights, than 0·02 p.p.m. The levels of molybdenum given in recent years range from 0·01 p.p.m. Arnon (1938) for tomato in water culture to 0·1 p.p.m. given by Piper (1942) in experiments with cereals and legumes, and by Sideris and Young (1946) with pineapple in water culture, and by Hayward and Long (1943), and Hayward *et al.* (1946) in sand culture experiments with peach and tomato. Warington (1946) found that although lettuce responded to addition of molybdenum there was no discernible difference between 0·001 and 10·0 p.p.m. Mo in water culture. 100 p.p.m. was toxic. It is likely that the practical limits lie between 0·02 and 0·2 p.p.m. molybdenum for most crops.

The levels recommended here for the micronutrient elements may, if *all* supplied at the upper limit given, induce symptoms of iron deficiency, if the iron supply is somewhat low, i.e. between 0·1 and 1 p.p.m. It is well known that many heavy metals may induce iron deficiency and the problem has been reviewed elsewhere by Twyman (1946), Wallace and Hewitt (1946), Hewitt (1948 b) (1949 a, c). Molybdenum may also modify the effects produced and it is therefore of the greatest importance to specify completely the nutrient composition so that the influence of micronutrient elements can be properly assessed and controlled.

Sources of Micronutrients

The compounds used to supply the known micronutrients are not of great importance. Boron is usually given as boric acid, but Mazé (1915) used borax and Brenchley and Warington (1927) tested 14 soluble and insoluble borates, all of which gave satisfactory results, except the cobalt salt which was slightly toxic to broad beans. Arnon and Grossenbacher (1947) supplied boron as the " Amberlite " complex in experiments with the use of synthetic resins. Löhnis (1937) found that boron as tourmaline was slowly available, and McHargue and Calfee (1933) and Muckenhirn (1936) showed that crushed " pyrex " glass was an available source of this element. Colwell and Cummings (1944) found that the uptake of boron by sunflower in water culture was independent of whether boron was given as boric acid or as calcium, sodium or potassium metaborates.

Manganese, copper and zinc are normally given as sulphates or chlorides, but in early experiments Salm-Horstmar (1851) used manganese carbonate. Guest and Chapman (1944) supplied manganese and zinc as well as iron as the humate salts at pH values of 7-9 and as inorganic salts at lower pH values. Arnon and Johnson (1940) also supplied manganese (and iron) as humate metals in experiments on the influence of pH on growth. The humate form of combination was first recommended by Horner *et al.* (1934) and the method of preparation is given in the Section on the supply of iron compounds. It is particularly suited to circumstances where precipitation of the metallic ions would lead to deficiency, i.e. in water cultures, especially where elevated pH values are expected. Horner *et al.* (1934) found that humate compounds of Al, Cu, Zn, Ni, Co, Hg, Ti, Cr, Mn and Fe can all be prepared and total concentrations of single or mixed metal compounds can be formed up to 20% of the total weight of humate. They suggested the inclusion of a mixture of humate micro-elements for nutrient solutions. Arnon and Grossenbacher (1947) supplied manganese (and also iron) as the " Amberlite ion " in experiments on the use of these synthetic resins as sources of adsorbed ions.

Molybdenum is normally given as sodium or ammonium molybdate and occasionally as molybdic anhydride, as for example by Hoagland and Snyder (1933), and Chapman *et al.*

(1937). Aluminium is usually given as the sulphate and gallium as the metal or as chloride or nitrate. Further indication of compounds used to supply microelement supplements can be had from the work of Hoagland and Snyder (1933) or Chapman *et al.* (1937), who also noted the spectrographic impurities present in each.

Organic Supplements for Inorganic Nutrient Solutions

It remains to consider whether the simple inorganic nutrient media described here are in fact adequate for all higher plants. The problem was investigated by Bottomley (1914) (1915) (1917) (1920 a, b, c) and Mockeridge (1924), who concluded that organic supplements were needed to secure full development in culture media by some plants. The stimulation obtained in Bottomley's experiments with *Lemna* were undoubtedly real, and at the time raised the question whether higher plants obtained all their requirements for growth from a purely inorganic medium. In some of these experiments Bottomley (1920 a) tested the effect of adding the ash of the stimulating organic fraction and observed no beneficial effect. The influence of the organic material on the availability of other nutrients, for example iron, however, was not considered. Wolfe (1926) critically examined the question and concluded that the beneficial effects were due to mitigation of toxicity from excessive nutrient concentration and lack of physiological balance. At the same time Clark (1926) also carefully re-investigated the problem employing a refined technique in which pyrex glassware, recrystallised salts, and triple distilled water were used and care was taken to exclude dust. Three principal salts and an iron compound only were present; no micronutrients were added. No stimulating effects whatever were observed due to addition of organic material.

In spite of this apparent finality the subject was re-opened by Ashby (1929 b). Ashby used a statistical technique with closely controlled environment and observed a catalytic response in growth rate to organic supplements which were not, however, essential for growth itself and which were ineffective after ashing. Bonner and Greene (1938) (1939) reported experiments in which they claimed that vitamin B_1 stimulated the growth of certain plants grown in inorganic culture media. Slow-growing plants or plants such as *Brassica* species with a low vitamin B_1 content in the leaves were benefited most, whilst fast-growing plants or those with a high natural B_1 content, such as tomato, were not affected. Arnon (1940) carefully repeated these experiments with the same species used by Bonner and Greene and used the rigorous technique devised to study the need of an inorganic micronutrient, but included at the same time all those already known to be required. No evidence of any stimulation was observed. Hamner (1940 b) obtained a similar negative result with vitamin B_1 and Ahlgren (1942) likewise obtained no response with thiamin supplied to grasses and clover.

Steinberg (1947 a, b) grew tobacco seedlings in sterile cultures to which inorganic salts and micronutrients (except molybdenum) were added. Addition of diffusates from bacteria produced growth abnormalities and no appreciable benefit occurred from the addition of vitamin compounds. The only work where a known supposedly complete inorganic medium has been used, however, remains that of Arnon (1940). Arnon and also Shive (1940 b) pointed out that as vitamin B_1 is needed for root growth and is synthesised in the green leaves, the controversy might be resolved in terms of the relative intensities of supply and demand, since some plants might be adequately self-supporting whilst others might be further stimulated by external supplements of organic compounds. Chesters and Street (1948) and Street (1950) again re-opened the question and claimed that lettuce grown in water culture or sand culture was stimulated by the addition of an infusion from leaf mould. No extra molybdenum was given although lettuce is known to be susceptible to molybdenum deficiency; oats Street, (1950) with low molybdenum needs, were unaffected; humate compounds from the leaf mould may also have materially improved the availability of iron in the water culture at the pH of 6·5 used. Even more recently, von Hausen (1948), Virtanen (1949) and Virtanen and von Hausen (1949 a, b) have reported that the cotyledons of pea and bean, and endosperm of cereals contain organic growth-promoting substances such as ascorbic acid, which they consider essential for nitrate assimilation during the seedling stage of growth. Glutathione and other reducing agents may replace ascorbic acid in this respect. In these experiments

the effects of replacing the deficiency of ascorbic acid, induced by removal of cotyledons or endosperm, by addition of molybdenum (which might have been eliminated at the same time as the ascorbic acid) were not examined, and the conclusion cannot therefore be accepted as final. Recently Hewitt *et al.* (1950) have found that molybdenum-deficient plants contain relatively lower amounts of ascorbic acid.

The interpretation of the somewhat familiar " cycle " of contradictory results in this field is thus still obscure. It is likely that more than a single explanation is involved. The accidental addition of a deficient micronutrient, the improvement of iron availability, the mitigation of toxic effects of excessive or unbalanced nutrient constituents, have not been consistently excluded in all the experiments, and may have provided reasons for some of the results. The possibility that root-stimulating compounds, or other metabolites, which for some reason were not provided in sufficient concentration by the aerial parts of the plants (or certain plants) as suggested by Shive (1940 b) may also account for others. In general, however, the wide experience of plant nutritionists indicates that all higher plants are able *ultimately* to make healthy growth in a purely inorganic medium, to which the addition of organic growth stimulants is unnecessary. The subject has been well reviewed by Chesters and Street (1948).

The Use of Radioactive Isotopes in Culture Experiments

Radioactive isotopes are distinguished from the usual elements by the special property of radioactivity. In all other respects they appear to have chemical properties identical with those of the non-radioactive isotopes.

Highly sensitive means are available to measure radioactivity and consequently minute concentrations of radioactive isotopes can be detected quantitatively. It is thus possible to " label " or " contaminate " a bulk of non-radioactive material with a small amount of active isotope which then behaves in the same chemical way as the whole, and brief consideration of their application is convenient at this point. The radioactive fraction is assumed to be absorbed or translocated by plants in similar proportions to the non-radioactive " carrier." By comparison between initial and subsequent radioactivity and total concentration, the origin, differential absorption and changes in distribution of the element during growth can be inferred.

Radioactive isotopes present in tissues can darken a photographic plate. The distribution of radioactivity, and thus of labelled material as a whole, can be determined in different histological regions in the manner indicated by Arnon *et al.* (1940), Stout *et al.* (1947) and Stout and Meagher (1948). Regions of growing plants can also be " scanned " for routine or immediate semi-quantitative purposes without interfering with the plant and rapid translocation may be followed immediately after application of a labelled element.

The purely chemical problems of nutrient purification and determination of efficiency of removal of microgram concentrations of impurities may also be greatly facilitated by the use of radioactive preparations to test different procedures.

Radioactive isotopes should be avoided if the experiment can be equally well carried out without them. The intimate contact between cell contents and atoms undergoing radioactive disintegration must be expected to lead occasionally to local cytological injury. Derangement of cell organisation and therefore of cell metabolism and permeability may result. There is, in fact, good evidence to conclude from the work of Scott Russell and Martin (1949) that concentrations of radioactive P_{32} used in many experiments can cause abnormal responses. Dry weight production and total phosphorus uptake by barley in water culture were adversely affected in this work by radioactive P_{32} at concentrations commonly used. Scott Russell and Martin have pointed out that the concentrations of the non-radioactive carrier may also affect results. Under " low salt " conditions relatively greater ion accumulation frequently occurs and for a given carrier ratio the absorption of the radioactive isotope may be materially increased. For certain elements, e.g. phosphorus, accumulation tends to occur at meristems and hence still higher concentrations of active material are produced in these regions ; any abnormalities induced here may be propagated during the subsequent life history of the plant.

A control experiment should always be carried out, without inclusion of radioactive materials, to check all quantitative effects before conclusions drawn from the " tracer " experiment are accepted. These criticisms are dealt with in detail by Scott Russell and Martin (1950 a, b).

It is neither possible nor appropriate here to attempt to review the literature on the detailed applications or conclusions relating to the use of radioactive isotopes in culture experiments. This section is intended only to refer to the possible applications of radioactive isotopes and to emphasise the criticisms that may be directed against their use. For detailed reviews and description of experimental technique and measurement, reference should be made to the following authorities :—Brewer (1941), Arrol (1946), Kamen (1947 a), Stout *et al.* (1947) and a book by Kamen (1947 b).

METHODS FOR THE RENEWAL AND APPLICATION OF CULTURE SOLUTIONS

The data in Tables 1 and 2 are sufficient to illustrate the frequency of solution changes in water cultures and rates of continuous renewal of solutions in sand and water cultures. Changes of solution are necessary to restore the concentrations of depleted nutrients, to maintain the nutrient balance and to avoid accumulation of undesirable waste products.

Intermittently Renewed Solutions

Frequent change of solution, although always desirable, is obviously the more important the smaller the culture vessels, the lower the concentration and the more crowded the plants. For special purposes, where accurate control of pH or nutrient concentration at low levels is required, large volume, stirred cultures containing only a few plants are advisable, and in addition to this general procedure, frequent checking (daily, or more often) and restoration of the critical variable are necessary. Eaton (1936) expressed preference for large volume, stirred cultures over small, continuously renewed ones, when problems of pH and concentration were to be investigated in water culture. This method has been used by Arnon (1937), Arnon and Johnson (1942), Arnon *et al.* (1942) and others for studies on pH and its relation to nutrient absorption, and by Parker (1927), Parker and Pierre (1928), Teakle (1929), Tidmore (1930) and Sommer (1936) for studies on phosphate uptake at low concentrations in solution, and by Chapman and Liebig (1940) for similar work on nitrogen uptake.

In experiments designed to study the essential nature of micronutrients, needed only in minute amounts, frequent change of solution is likely to delay the appearance of symptoms owing to the continual introduction of fresh traces which prolong the period of normal growth. cf. for example McMurtrey 1929. It is equally important, however, to avoid depletion of the nutrient with respect to other essential macronutrients or stagnation effects, and the only satisfactory method is to use a solution sufficiently purified that the effect of reasonably frequent renewal, say weekly, as a minimum for a 1-L. water culture is negligible. If search is to be made for micronutrients not yet identified, comparisons between frequent and infrequent changes might be advisable.

The suggested frequency of renewal for fairly small volume water cultures varies considerably. Shive and others at New Jersey adopted a twice weekly change as the standard frequency. Brenchley (private communication) considers that two-week intervals are satisfactory for many plants and suggests weekly intervals for some larger ones. Others (Table 1) have suggested longer intervals, but for 600 ml cultures the twice weekly method seems most satisfactory and weekly intervals might be adopted for 1-2 L. cultures. Large tank cultures of 40-100 L. or more require change about every 2-4 weeks depending on plant species, density and season. However, as water is continually lost by transpiration and evaporation, the deficit must be made good by daily additions of water during the interval between renewal, in order to prevent the development of high salt concentrations.

Close control of pH is more difficult in sand cultures as noted previously owing to the restriction of diffusion and the greatly reduced volume of fluid immediately surrounding the roots, however large the culture. The same limitation obviously applies to control of low concentration of nutrients. Intermittently watered sand cultures are thus less suited to

precise work concerned with problems of pH or nutrient concentrations, except where pH stabilisation is provided by nutrient composition, but this is not easily achieved.

Intermittent application of nutrient solutions to sand cultures is, however, an entirely satisfactory procedure for the study of deficiency effects, nutrient ratios and inter-relationships and salt effects, provided that daily (or if possible twice daily) applications of an adequate quantity of solution are given.

The magnitude of the requirement for an element may depend on many factors, including light, concentration of other elements and the particular plant used. Thus Chapman and Liebig (1938) found that 0·7 p.p.m. N allowed normal growth of orange seedlings in sand cultures flushed at hourly intervals, but produced nitrogen deficiency if the intervals were 8 hours. Tomato, on the other hand, made progressively more growth as the concentration was raised to 70 p.p.m. N, even with hourly flushings.

It is desirable that excess solution should be given to the surface of the sand so that part of the solution previously added is displaced from the base of the culture and drains freely away. For seedlings, or during cool weather, less frequent applications are satisfactory so long as the *surface* of the sand is damp, thereby indicating that there is no marked increase in water tension. " Bottom watering " by means of saucers adopted by Nightingale *et al.* (1932), Davis *et al.* (1934) and Nightingale and Blake (1934 a) may provide more constant water tension and concentration of nutrients, but is seldom used. In the opinion of the writer these " advantages " are outweighed by the stagnation, waterlogging, lack of aeration and poor growth conditions so incurred. Sub-irrigation methods also have the disadvantage of not securing complete flushing of the sand and require more careful control and handling than surface application methods. Eaton (1942 b) stated a firm preference in favour of surface application and considered that upward or sub-irrigation was usually unsatisfactory. He suggested that renewal of the solution should be made at hourly intervals.

Leaching

Although daily (or frequent) watering of sand cultures supplies adequate moisture, additional leaching is necessary to maintain the favourable growing conditions. Leaching should be frequent where rapid evaporation and translocation cause rapid alteration in concentration or where the nutrient composition or pH tends to vary widely as for example with ammonium nitrogen. Leaching has been widely used since the early experiments of Wallace (1924 (1928) who leached tree and soft fruit cultures every two weeks with water only, and this practice contrasts strongly with the earlier undrained and surface-sealed sand culture methods used by McCall (1916 a, b), Shive (1918 a) and others cited in the section on sand. Wallace (1928) found that strawberry plants were especially sensitive to omission of leaching and were killed if it was not done, whereas blackcurrants and gooseberries were able to continue growth. Gooseberries, on the other hand, were highly sensitive to waterlogging, which rapidly caused leaf scorching and defoliation. Leaching once a week was adopted by Weinberger and Cullinan (1936), Cullinan *et al.* (1938) and Brown (1945), where high concentrations of nutrient were given for peach trees, and by Heller *et al.* (1940) in salinity experiments ; Roy and Gardner leached once every two weeks with 30 L. over a period of 8 hours. Alben *et al.* (1942) leached pecan sand cultures at frequent intervals.

Even where constant renewal of solution is practised, additional leaching has been considered necessary. Thus Spencer and Shive (1933) flushed the sand weekly with water in addition to solution renewal at the rate of 1 L/day. They also broke up the surface of the sand once a week. Tomato cultures supplied with 2 L/day were leached twice weekly by Nightingale *et al.* (1931). Beckenbach *et al.* (1936) leached with water at the rate of 1 L/day each evening in an experiment where constant renewal was used. Clark (1941) leached the sand with water every 4 days in spite of a solution renewal rate of 12 L/day. Tiedjens and Blake (1932) leached apple tree sand cultures weekly in addition to solution application every 2 hours during daylight and once at midnight. The importance of periodical leaching in some instances at daily intervals, even in constantly renewed cultures, is emphasised by Shive and Robbins (1942) and Robbins (1946).

Re-use of Nutrient Solutions

The fact that absorption of ions from, and infusion of organic products into nutrient solutions is continually occurring naturally limits the extent to which a solution, that has passed through a sand culture, can be re-used. For reasons of economy, however, especially with high rates of renewal, re-use is frequently practised, either for a small number of cycles or for longer periods with periodical supplementation of one or more nutrients. Thus Cullinan *et al.* (1938) collected the leachate from the base of the pot, made it up to the volume applied and re-used it once only. Batjer and Degman (1940) adopted a similar method, but re-used the original solution for a week before discarding it. Mitchell (1939) grew *Pinus strobus* in irrigated sand cultures and returned the drainings to the reservoir where the volume was made up to its original level. Tiedjens (1934) supplied solution at 10–24 L/day and re-used it for three days before preparing fresh nutrient. Clark (1941) in tests on pH effects supplied solution continuously at 12 L/day. The drainings were returned to the reservoirs to which an extra 6 L. of fresh solution were added every 3 days. The solution was checked and adjusted for pH daily. Eaton (1931) re-used solution from large sand beds for a period of 5 days or until 50 gallons had been lost. Chapman and Liebig (1940) used large reservoirs of 100 L. for three 2-gallon cultures with citrus. The excess fluid from each irrigation was returned to the solution in the reservoirs which was replaced every month. Daily checks and adjustments were made for nitrogen level. The low level of 0·3 m.eq./L PO_4''' was adequate for the duration of the experiment in this work. Shive and Robbins (1942) found that the " spent " solution from continuous renewal cultures was non-injurious to tomatoes and was able to produce healthy, vigorous growth. They considered that such solutions could be used several times over without bad effects.

Alternating Solutions

A special type of solution change is that of alternating solutions. This method has been used to grow plants in the presence of compounds that precipitate each other, for example salts of aluminium and phosphates. By alternating the plants between complete solutions deficient only in either one of the reacting salts, uptake of both is ensured. McLean and Gilbert (1926) and Gilbert and Pember (1935) grew many crops in water cultures where the solutions were alternated for 3½-day periods between a complete nutrient and a similar solution devoid of phosphate containing aluminium, or else between complete nutrient solutions with and without aluminium. Liebig *et al.* (1942) carried out similar experiments with citrus. Richards (1945 unpublished) found that chlorosis in flax was prevented and good growth obtained by alternating the plants between a complete nutrient and a phosphate deficient solution for 16 hours twice weekly.

Continuous Renewal of Solution

It may be thought that the ideal means of maintaining nutrient composition is to renew the solution at such a rate that changes in concentration are negligible. The suggestion appears to have been made by Nobbe (1865) (1868). This procedure has been widely adopted as shown in Tables 1 and 2 and in examples discussed previously. There are certain limitations, however, to be borne in mind in the application of this principle. First, continuous renewal of solution involves considerable increase in equipment and maintenance ; the bulk of solution, if it is to be really effective in its purpose, may be considerable as, for example, was found by Clark (1933) (1941), Clark and Shive (1934), Nightingale (1934), Tiedjens (1934), with rates up to 36 L/day. Aeration in sand cultures may be reduced by partial waterlogging, although Nightingale (1934) and Addoms (1937) found that high rates of renewal can be tolerated. Robbins (1946) pointed out that a coarser particle size might be needed. On the other hand. it is probable that this method is the most effective for *sand cultures* where close control of pH or nutrient ratios, or low nutrient concentrations are needed. Details of much comparative work on the use of static and flowing solutions has already been given in the Section on sources of nitrogen. Continuous renewal methods are also useful for absorption tests when small containers are used for short experiments such as those of Stahl and Shive (1933).

Continuous renewal might also be expected to delay the onset of deficiency effects where a micronutrient element has been omitted by accentuating effects of slight contamination. Chandler (1941) (1944) and Reeve and Shive (1944), however, found no difficulty in this respect in the production of boron deficiency in sand and water cultures.

Methods

The Mariotte's Bottle

Schloessing (1899) introduced the Mariotte's bottle method for constant rate of flow. The arrangement used by Schloessing for sand cultures is shown in Figure 5 A. Care was taken to secure even distribution into 40-L. vessels and rates up to 10 L/day were used. The Mariotte's bottle principle has been used in many of the subsequent arrangements described. It is reliable, convenient, self-acting, easily adjustable and equally effective for large or small reservoirs, if certain accessories are included.

The Mariotte's bottle comprises a container sealed from the atmosphere except for an inlet that is arranged to permit the entry of air at almost the lowest level below the surface of the liquid, and an outlet for fluid that can be either by siphon over the top aperture or direct from the base. The rate of delivery is then determined (theoretically) only by the " head " between the level of the air inlet and the level of the fluid outlet and the diameter of the outlet tube, and as each of these is easily altered, a simple control of rate is possible.

Probably the simplest equipment for continuous solution renewal was that used by Behrens (1928). This comprised a simple Mariotte's bottle where the solution was delivered by a siphon so that a basal tubulure was unnecessary, as shown in Figure 5 D. The head is altered by raising or lowering either the air inlet or the solution outlet. Shive and Stahl (1927) and Shive and Robbins (1942) also devised a simple and much-used technique. This consisted of the arrangement shown in Figure 5 C. The rate of flow is governed by the bore of the capillary tube. A bore of 0·8 mm. gives a rate of about 2 L/day and a bore of 0·5-0·6 mm. a rate of 1 L/day according to Robbins (1946). The capillary tube is bent to an S shape and fits into the 2-quart reservoir inverted over a glass " nappie " through a notch cut in the rim. By making this notch a little smaller than the size of the tube, the latter is gripped by the weight of the jar and is held in any required position. Algal growth in the capillary is avoided by blackening the outside and the tubes do not get readily blocked up. The method of Shive and Stahl (1927) is only suitable for rates of flow up to about 4 L/day, otherwise frequent refilling of the reservoir is needed.

Several constant-flow arrangements whereby a small constant-level vessel supplied by a large reservoir is used to supply solution to one or more delivery tubes have been described by Trelease and Livingstone (1922), Allison and Shive (1923), Johnston (1927) and Tyner (1935). The small vessel is continually replenished from a Mariotte's bottle device. This method gives better control, eliminates errors due to changes in temperature and also allows several cultures to be maintained by one reservoir. As two reservoirs can be arranged to supply the same constant-level vessel the change-over can also be accomplished without any interruption of the flow. Mehrlich (1935) devised a somewhat elaborate system to give refined control of the rate of flow, change-over from the emptied reservoir to the waiting full one being automatic. The arrangements used are shown in Figures 5 E, F, H, I. Equipment used by Johnston (1927) was also arranged to allow rapid change-over between empty and full reservoirs and was able to cope with high rates of flow. The rate was controlled by the siphon exit which was the limiting aperture. Gallegly and Walker (1949) placed an intermittent siphon in the base of sand culture pots to regulate the escape of nutrient applied by continuous flow.

Equipment involving a large amount of glassware, numerous clips and intermediate vessels, etc., is less suitable for use if problems relating to micronutrients are concerned. For such work slight loss of precision in rate, and elimination of any unnecessary equipment is preferable to risk of contamination and the arrangement suggested by Behrens (1928), Figure 5 D, provides the nearest approach to this criterion. The necessary modifications to allow of easy and uncontaminated operation for micronutrient-deficiency experiments would

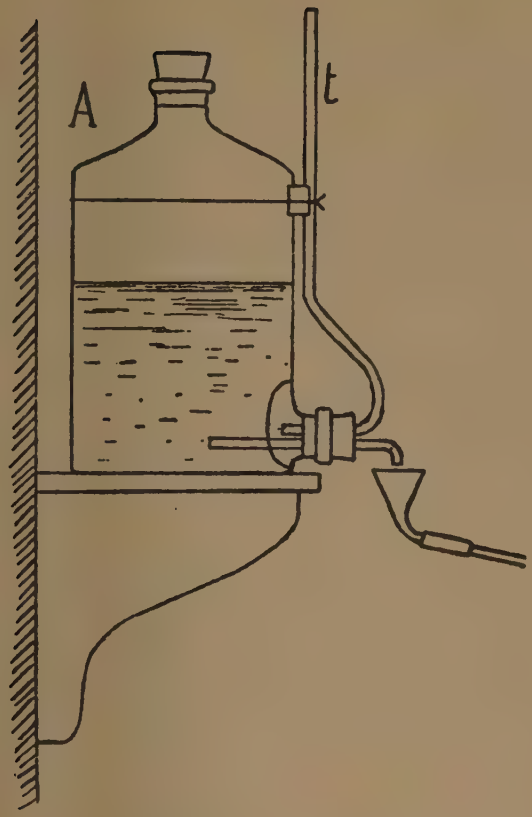


FIGURE 5A(I) (*above*)

Constant rate of solution renewal in sand culture (*Schloessing*, 1899)

A = Mariotte 12-litre jar or bottle.
t = Air inlet tube.

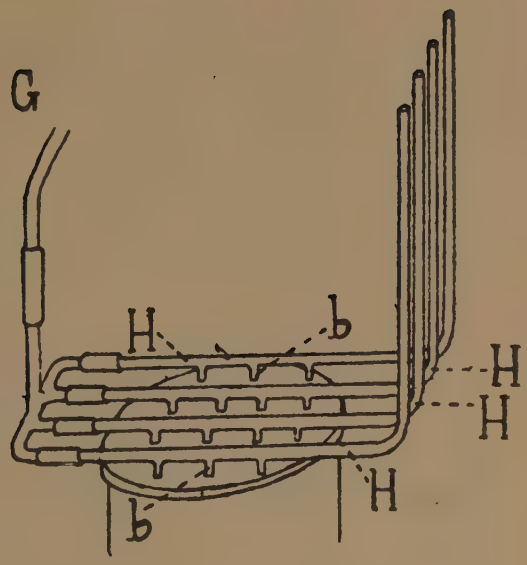


FIGURE 5A(II) (*top right*)

Multiple distributor (*Schloessing*, 1899)

G = Supply tube from Mariotte reservoir.
H = Tubes placed parallel above the sand (or soil).
b = 20 dispersal branches (capillary tubes) going down almost to the sand level.

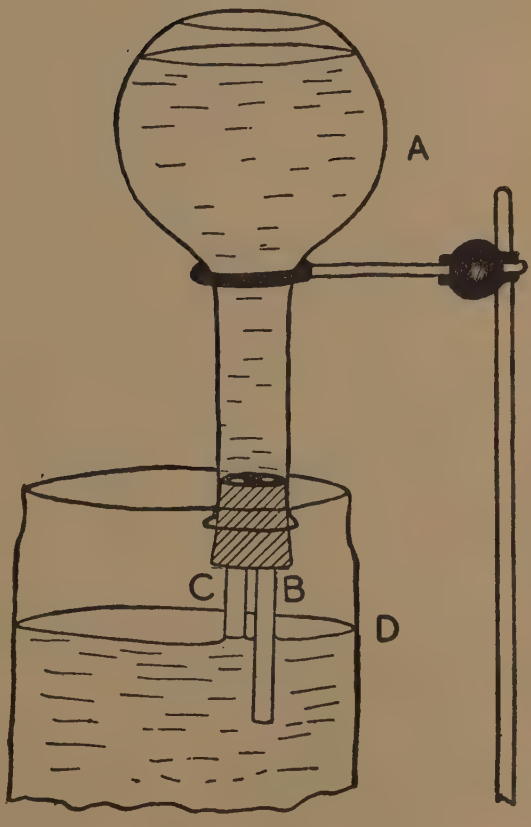


FIGURE 5B (*right*)

Constant-level device for water culture (*Clark*, 1916)

A = Inverted reservoir.
B = Solution delivery tube.
C = Air inlet.
D = Solution level.

include a glass stopcock on the delivery siphon, an additional inlet where air pressure could be applied to charge the siphon, a stopcock on the air line and one on the tube for attachment to a refilling reservoir, so that suction could be applied to the extra inlet to fill the Mariotte's bottle.

Zinzadze (1935) used an annular valve (Figure 5 G) that could be varied by the insertion of the central plunger to control the rate of flow into water cultures. The level in the annular valve was determined by the air inlet of the Mariotte's bottle. The valve could be closed by pressing the plunger down to a short rubber tube to seal the outlet. The entry and outlet for the solution in the culture vessel was made in the side so that plants could be removed independently of the arrangement of the tubing.

Constant Level Cultures

A few workers, including Clark (1916) and Haas (1932 b), have devised constant level cultures in which solution is only renewed when the level falls due to evaporation and transpiration. Anderssen (1926) used this procedure to avoid filling the culture vessels, when it was desired to keep a part of the root system in humid air and still maintain a flow of solution. Haas (1932 b) used a dilute solution of ferric citrate to maintain the level in shallow pans used to grow Citrus and thus supplied iron at a rate comparable with that of water. The principle is shown in Figure 5 C.

Wick Cultures

Wick cultures deliver solution through the capillary action of a wick and were used by Raines (1937) (1939 a). The " wick " may be of blotting paper, cotton wool, asbestos or other material. Such methods appear to have little to commend them. The wicks easily become fouled and clogged and high rates are not possible. A wick method of automatic irrigation was described by Norem (1936) to maintain an even water tension in sand cultures. Such techniques are not suitable for micronutrient work and for other purposes tensiometers, automatic irrigators and the like appear altogether superior in spite of the need for extra equipment.

Atomised Culture Solutions

A special example of solution renewal and its relation to root aeration is that where the roots of the culture plants are not immersed in sand or water but are only sprayed with the culture solution. This method was first devised by Barker (1922) and was found by Pearse (1937) (1940) to improve the growth of apple trees over that obtained in normal water culture. The method was developed a stage further by Carter (1942) who used a stream of tap water (under pressure) to supply water. The nutrients were automatically incorporated by injection from a cylinder in a by-pass, controlled with a needle valve and the diluted solution was sprayed against the roots of pineapple plants. It was claimed that this method was at least equal in results to orthodox water cultures and facilitated root examination. Pearse (1937) (1940) originally adopted the technique owing to difficulties in the provision of adequate aeration for the tree cultures. Klotz (1944) used a de Vilbiss atomiser to spray a " mist " on to the suspended roots of culture plants and thus circulated the nutrient solutions without dilution. A glass wool filter was used to prevent fouling of the atomiser jet.

Tensiometers and Methods based on Water Loss

In some experiments it may be necessary to maintain a constant water tension in the rooting medium. The moisture content can be determined either by weight or by a suitable tensiometer. Although restoration of weight to a predetermined value may be a suitable means for some purposes, it is tedious when several pots are involved, especially if frequent restoration of moisture is required. Also it does not take account of the gain in fresh weight of the plant during growth, unless allowance is made periodically. However, a few methods for maintaining moisture content based on change in weight have been described by Steinberg (1930) and Johnson and Atkin (1939). These are somewhat elaborate and complicated and not readily applied to the maintenance of several pots at once. The equipment used by Steinberg was electrically, and that of Johnson and Atkin mechanically operated. Several tensiometers

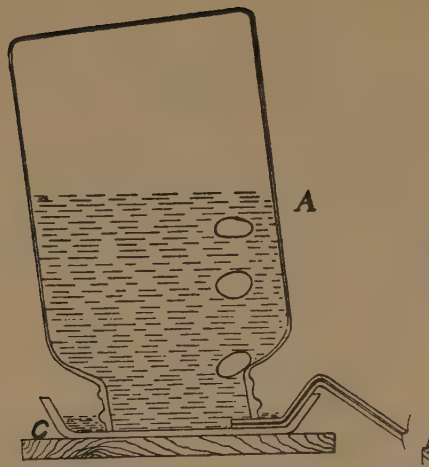
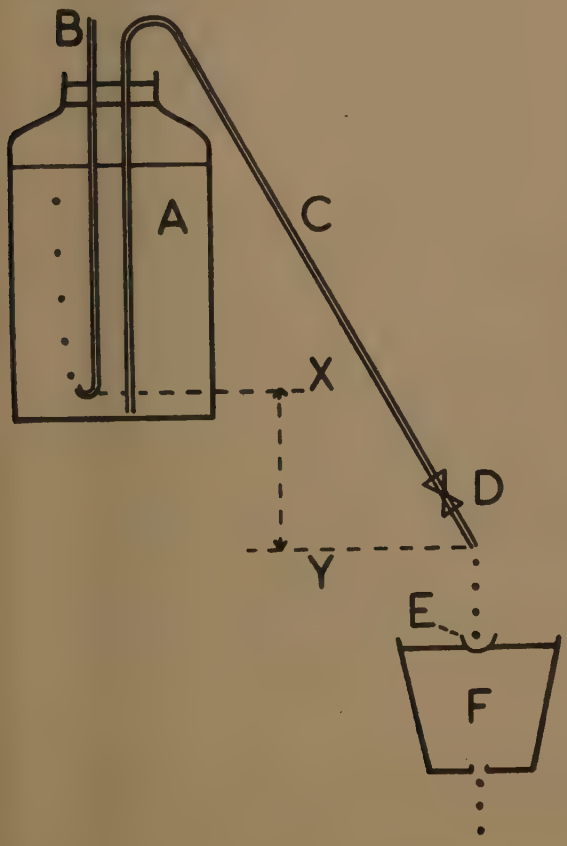


FIGURE 5D (*below*)

Constant rate of renewal in sand culture (*Modified after Behrens*, 1928)

A = Reservoir sealed at top.
B = Air inlet.
C = Siphon delivery tube.
D = Stop-cock (optional).
E = Small dish to aid distribution of solution.
F = Pot.
X-Y = Head.



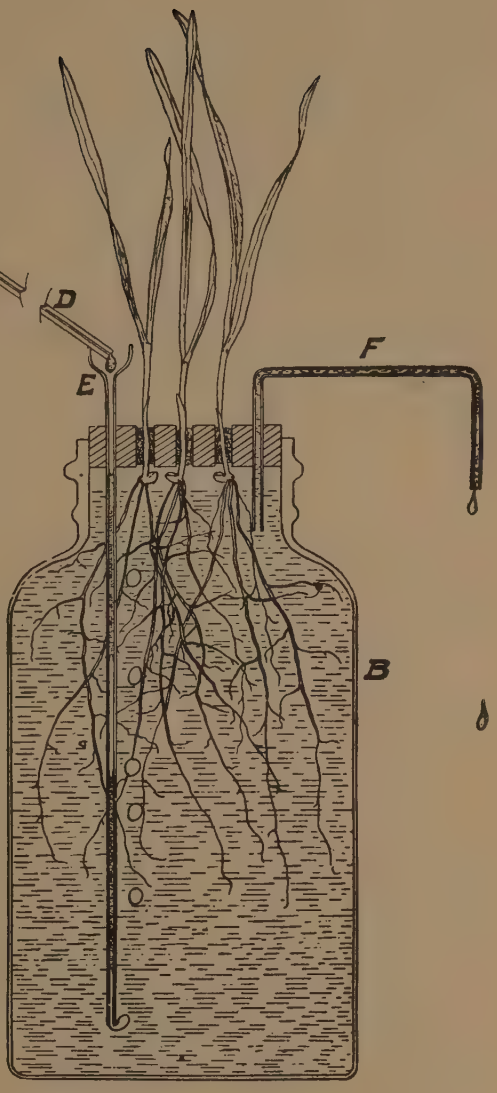


FIGURE 5C (*above*)

Constant-renewal and constant-level in water culture. (*Shive and Stahl*, 1927)

A = Constant-level reservoir.
B = Culture jar.
C = Flat glass dish or " nappy."
D = Siphon (0·5-1·0 mm. according to rate of flow, adjusted by raising or lowering outlet).
E = Inlet tube with entrained air bubbles.
F = Outlet tube (in a later type used by Shive and Robbins (1942) the outlet tube is extended from the base of the vessel and the exit is turned upwards).

designed for use in soils have been described, but they are not readily applicable to coarse substrates like sand. Heath (1929) constructed porous filter candle tensiometers for use in sand cultures. They were calibrated by tests in sand of known uniform moisture capacity and attained equilibrium in 5-15 minutes with an accuracy of 1-2% in sand with a moisture capacity of 26·1% and graded between 1 and 0·02 mms. Richards and Blood (1934) described an improved auto-irrigator which was a simple " home-made " type, but this was only tested for soils. A useful list of references was included. Kolodny and Robbins (1940) used an auto-irrigator technique to supply nutrient in experiments on the absorption of potassium from bentonite after finding that drip culture application rapidly leached out the adsorbed potassium.

A reliable and accurate auto-irrigator, based on a tensiometer, for use in sand culture as well as soil has been designed by Anderson (1944 b). A single aspirator arranged as a Mariotte's bottle reservoir maintained a constant quantity of water in several individual watering units. Each of these was activated independently by a tensiometer in a battery of cultures, which could be double-compartment pots holding sand over solution. The water content of the sand or soil was maintained between prescribed upper and lower limits by activation of the irrigator at the required minimum moisture content and delivery of a set quantity of fluid. The irrigator was largely independent of temperature changes in the greenhouse, but the porous candles had to be specially made in order to have the appropriate porosity. It is necessary to refer to the original paper for details of the full specification.

Distribution of Solution

Uniform distribution of solution requires more attention in sand than in water cultures. Behrens (1928) placed a porcelain dish, embedded just below the region receiving the drips, to act as a distributor. A watchglass, convex side up, was used for a similar purpose by Allison and Shive (1923) and a glass tube embedded in the sand was used by Tyner (1935). Pirschle (1929) used a 5-armed, multiple jet for the same purpose. Zurbicki (1933) constructed a balanced tilting device, designed to alternate solution at short intervals between a pair of sand cultures so that the solution was prevented from dripping continually on the same spot but was shot out across the surface of the sand and spread out by glass plates. It had the disadvantage of being delicate to adjust for equal volume delivery and tended to become blocked by air locks.

Distribution of the fresh solution in a water culture is easier to accomplish, especially if additional aeration is provided, as this produces effective stirring. The direction of flow in Shive and Stahl's (1927) method was *along* the bottom of the vessel, and was later changed from bottom to top by Shive and Robbins (1942). This direction was also used by Trelease and Livingstone (1922), Teakle (1929) and Zinzadze (1935). Johnston (1927) and Pirschle (1929) arranged the flow in the reverse direction, i.e. from top to bottom, and Marsh (1935) compared the effects on growth of soya bean of bottom and top draining. Bottom draining was appreciably superior. Maskell and Evans (private communication) devised a neat method to circulate nutrient solution in aerated water cultures by a miniature air lift immersed in each culture as shown in Figure 5 J. This device permitted good growth of cacao. Humphries (1950) combined solution renewal and aeration under controlled conditions shown in Figure 5 K.

Pouget and Chouchak (1912) used a water suction pump to circulate the solution in a water culture and arranged for the solution to flow past the roots of the culture plants in a small vessel and circulate back through a large reservoir. The equipment is of interest only.

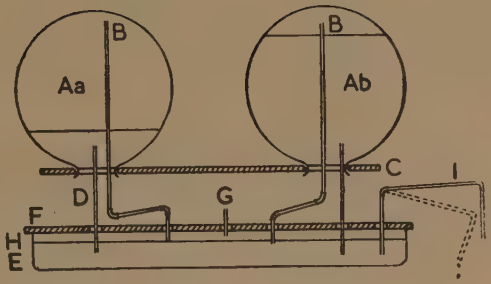
Pearse (1937) described two arrangements for circulating and aerating nutrient solution for apple trees grown in water cultures, as shown in Figure 6 E. One was operated by air pressure and the other by a suction pump. The spray method worked by the pressure airlift gave better results than the other method where the roots were immersed or than a third method where a solution was aerated but not circulated.

FIGURE 5E

Accurate control of solution renewal independent of temperature changes and providing automatic change-over of reservoir (After Mehrlich, 1935)

Aa = Solution reservoir in use.
Ab = Solution reservoir in reserve.
B = Air inlets.
C = Support.
D = Solution outlets.
E = Constant-level reservoir.
F = Lid.
G = Opening to air.
H = Level of solution.
I = Delivery tube controlling rate of flow by altering angle.

Note.—Levels of the air inlets from Aa and Ab into E are adjusted to prevent the contents of Ab being used until Aa is empty and *vice versa*.



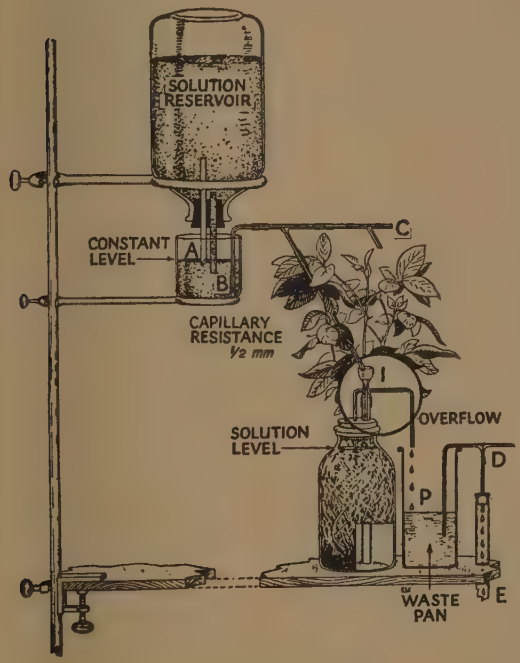


FIGURE 5F

Regulating the flow of liquid into a culture vessel by capillary tube (After Allison and Shive, 1923)

A = Air inlet.
B = Solution drops through B until level in lower vessel rises over the opening of A.
C = Main conducting tube.
I = Small funnel.

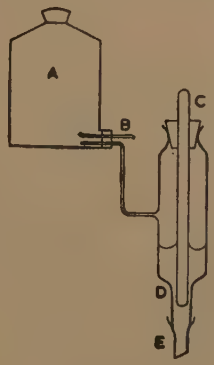


FIGURE 5G

Regulating the flow of liquid into a culture vessel by annular valve (After Zinzadze, 1935)

A = Reservoir sealed at top.
B = Air inlet.
C = Glass rod.
D = Annular valve.
E = Rubber sleeve to close, when C is lowered.

FIGURE 5H

Accurate control at high rates of delivery (After Trealease and Livingstone, 1922)

A = Air inlet tube controlling head of solution flowing into L.
B = Siphon tube with stop-cock.
C = Connection to suction pump with stop-cock.
D = Delivery tube (small bore).
E = Overflow tube maintaining constant level in L.
F = Thistle-tube receiver to bottom of culture vessel.
G = Waste tube controlling level in culture vessel.
H = Stop-cock controlling air inlet to reservoir.
I = Tube for refilling upper reservoir.
L = Constant-level device.
O = Lower reservoir.
R = Upper reservoir.
V = Culture vessel.
For delivery B, H are open and C closed, and *vice versa* to refill.

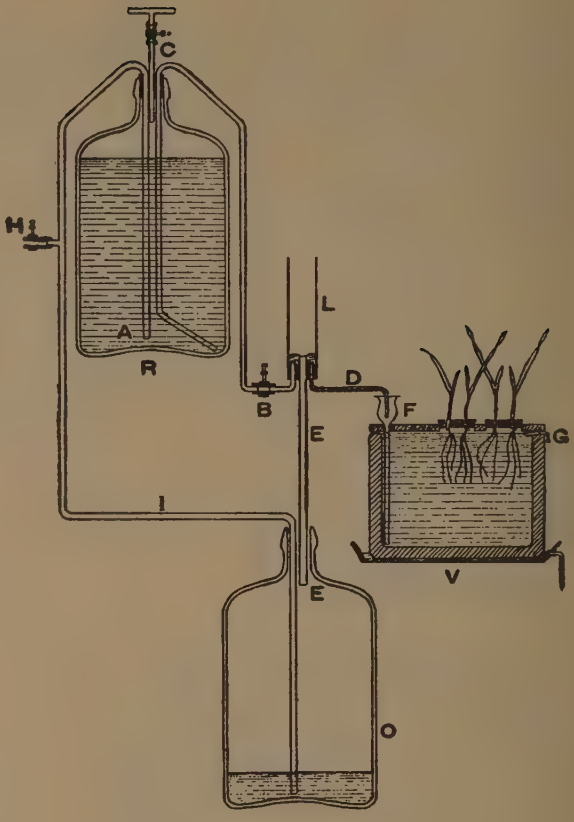
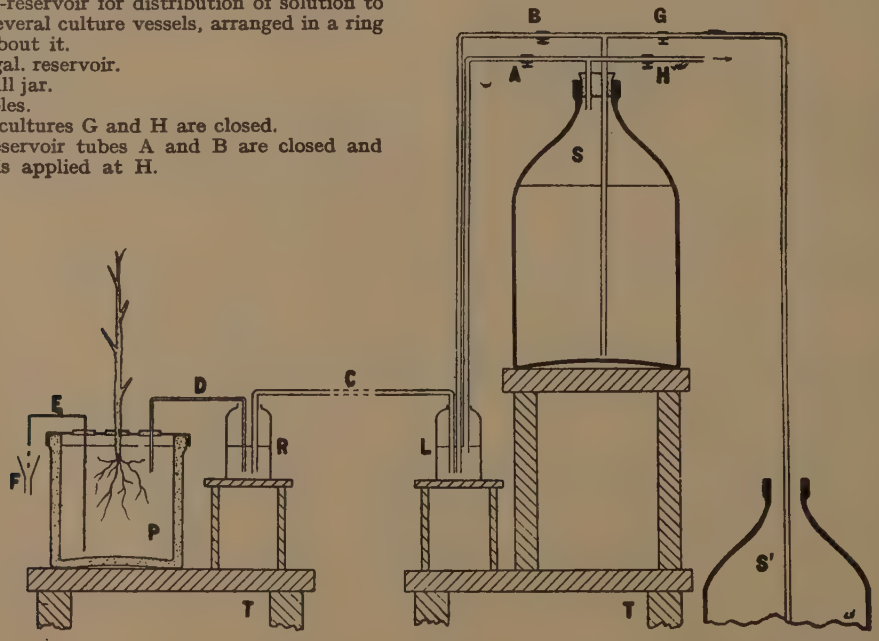


FIGURE 5I

Designed to supply 6 water cultures at 7 litres per day each (Johnston, 1927)

A = Air inlet tube and stop-cock (tube of 7 mm. bore with lower end ground at 45°).
B = Siphon tube and stop-cock.
C, D and E = Siphons.
F = Waste tube.
G = Refill tube and stop-cock.
H = Tube to suction pump and stop-cock.
L = Constant-level jar (level maintained by outlet from tube A. When level drops below outlet, air enters S and solution siphons through B into L).
P = 5-gal. plant container.
R = Sub-reservoir for distribution of solution to several culture vessels, arranged in a ring about it.
S = 10-gal. reservoir.
S¹ = Refill jar.
T = Tables.
To supply cultures G and H are closed.
To refill reservoir tubes A and B are closed and suction is applied at H.



Metering Pumps and Other Equipment

These have been devised by DuBuy (1939) and Rains (1939 b) to deliver small volumes automatically to several cultures. They involve moving parts and do not appear to have important applications or advantages over Mariotte's bottle arrangements for continuous supply of solution.

Two devices described by Blake (1945 b) (1946) merit attention here. The first was a method to record and compare solution concentrations. The equipment, which was electrically operated, measured conductivity and provided for automatic regulation of concentration. It might thus be adaptable to culture experiments in such a way that testing the composition of an effluent was used to regulate the concentrations of solution to be applied. The second device was an electromagnetic liquid-flow control valve that might be of use in large-scale sand cultures, to control application in irrigated installations.

Miles (1947) has described a method used for large-scale irrigation beds whereby the solution was injected as a concentrate into a continuous (or intermittent) flow of tap water. This permitted the preparation of the equivalent of large volumes of solution at longer intervals, and the use of smaller solution reservoirs. The method was used for hydroponic installations, but might find application where it was desired to grow plants with high rates of solution renewal or irrigation, provided use of tap water and elimination of contamination were not limiting factors. The injector pump was automatically activated by the flow of mains water and the dilution factor could be varied by changing the stroke of the pump.

Solution Renewal in relation to Aeration in Water and Sand Cultures

Arrangements where the Mariotte's bottle is used have the advantage that the bubbling of air which replaces the solution in the reservoir partially aerates the solution. In water cultures where the drips are guided down a funnel into the solution air is also entrained by the descending column of liquid and this provides an additional source of aeration. Continuous solution renewal has in fact been regarded at times as a substitute for forced aeration and may be adequate for some plants, for example oat plants grown by Stahl and Shive (1933). Shive (1923) found, however, that three 21-day-old soya bean plants removed practically all the oxygen from a 2-L. water culture in 12 hours even when solution was renewed at 2 L/day.

Allison (1921) examined the influence of aeration and solution renewal on soya bean grown in sand and water cultures with the following results :—

Method	Static Water Culture	Static Sand Culture	Static Water Culture aerated	Flowing Water Culture	Flowing Sand Culture
Relative dry wt. ...	1·00	1·80	2·04	2·05	2·23

Allison (1923) made a similar comparison for the growth of barley and soya bean in water cultures with aeration or solution renewal or both. The following data were obtained :—

Method	Allison (1923) O_2 tension p.p.m.		Relative dry weight	
	Barley	Soya Bean	Barley	Soya Bean
Static culture renewed twice weekly	1·41	1·85	1·00	1·00
Ditto aerated	3·30	4·08	1·15	1·08
Continuous renewal 1 L/day	2·05	2·42	1·29	1·03
Ditto aerated	3·08	5·01	1·29	1·35

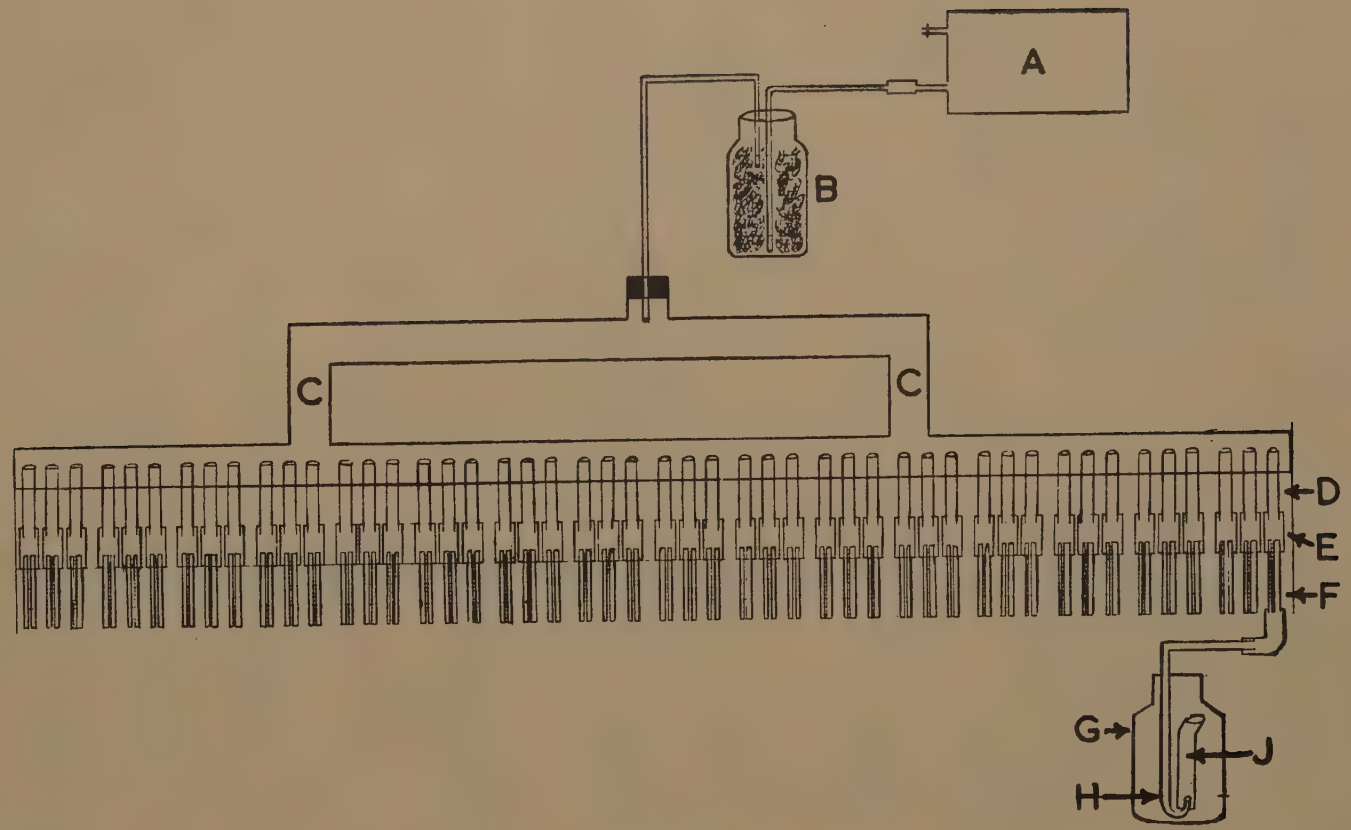


FIGURE 5J

Multiple aerator equipment and device for circulation of nutrient solution in water cultures of cacao (*Evans and Maskell* 1950, *private communication*).

A = Air pump.
B = Air filter (glass wool).
C = Manifold (2″ gas pipe)
D = Brass side tubes.
E = Rubber tube joints.
F = Capillary resistances.
G = Culture vessel with air-circulating device.
H = Air outlet.
J = Glass tube circulator open at both ends, supported on H.

Allison and Shive (1923) made a further comparison of solution renewal and aeration for sand and water cultures with soya bean and attempted to force air through the sand cultures from the base by means of a pump. The relative growth data are given below :—

Allison and Shive (1923)

Method	Sand Culture	Water Culture
Intermittent renewal twice weekly ...	100	57
Ditto aerated	97	47
Continuous renewal	123	87
Ditto aerated	156	127

The superiority of the solution renewal in sand culture was noteworthy ; there was usually a response to either aeration or solution renewal in the water cultures and a further response to both combined.

Irrigation Techniques specially adapted for Sand Cultures

A more satisfactory alternative to weight or tensiometer irrigation for sand cultures is comprised in the automatic irrigation techniques described by Eaton (1936) (1941 a), Chapman and Liebig (1938), Gauch and Wadleigh (1943) and Arnon and Grossenbacher (1947). These appear well suited to large-scale operations ; they are automatic in operation and controlled by electrically-controlled (standard pattern) time switches and clocks. The methods are useful for work where the constancy of composition or pH is required, as for example in the experiments of Chapman and Liebig (1940) and Guest and Chapman (1944). The excess nutrient is returned to large reservoirs and is then re-circulated after mixing with a relatively large residual volume of solution in the reservoirs. Periodic restoration of pH or concentration is easily achieved, and the circulation is obtained by causing the drainage fluid to re-enter the reservoir far away from the point of exit. The details often required to be closely specified and for this information further reference should be made to the original papers. The general arrangement and equipment are described here and illustrated by diagrams in Figure 6 to assist in deciding their value for a particular purpose.

The arrangement used by Chapman and Liebig (1937) (1938) and Chapman (private communication) is shown in Figure 6 A. It will be seen that the solution moved downwards through the sand in the normal manner. The solution was raised to a distributor by a simple airlift. The distributor supplied up to 3 cultures of 2-gallon capacity or a single 25-gallon ash bin at one time from a single reservoir. The reservoir was of 100-L. or 270-L. capacity and was made by sealing a plate over the end of a large glazed drain pipe which was painted with bitumen inside. The sand culture was surmounted on the lid of the reservoir. The airlift was operated by compressed air and controlled by a time switch mechanism. The data on pumping time, volume of solution and height raised were given as follows :—

	Height pumped	Time	Volume of air	Volume of liquid
2-gallon culture ...	20″	5 mins.	20·8 L.	10·6 L.
25-gallon „	36″	10 „	65·0 L.	28·0 L.

Use of a small compressor was possible if the timing was arranged so that each unit or group of units was irrigated serially. Prolonged irrigation, fast enough to ensure a slight excess of liquid on the surface of the sand, was adopted to obtain even flushing. The air lift provided a well-aerated solution adequate for sand cultures, including citrus, when irrigated hourly.

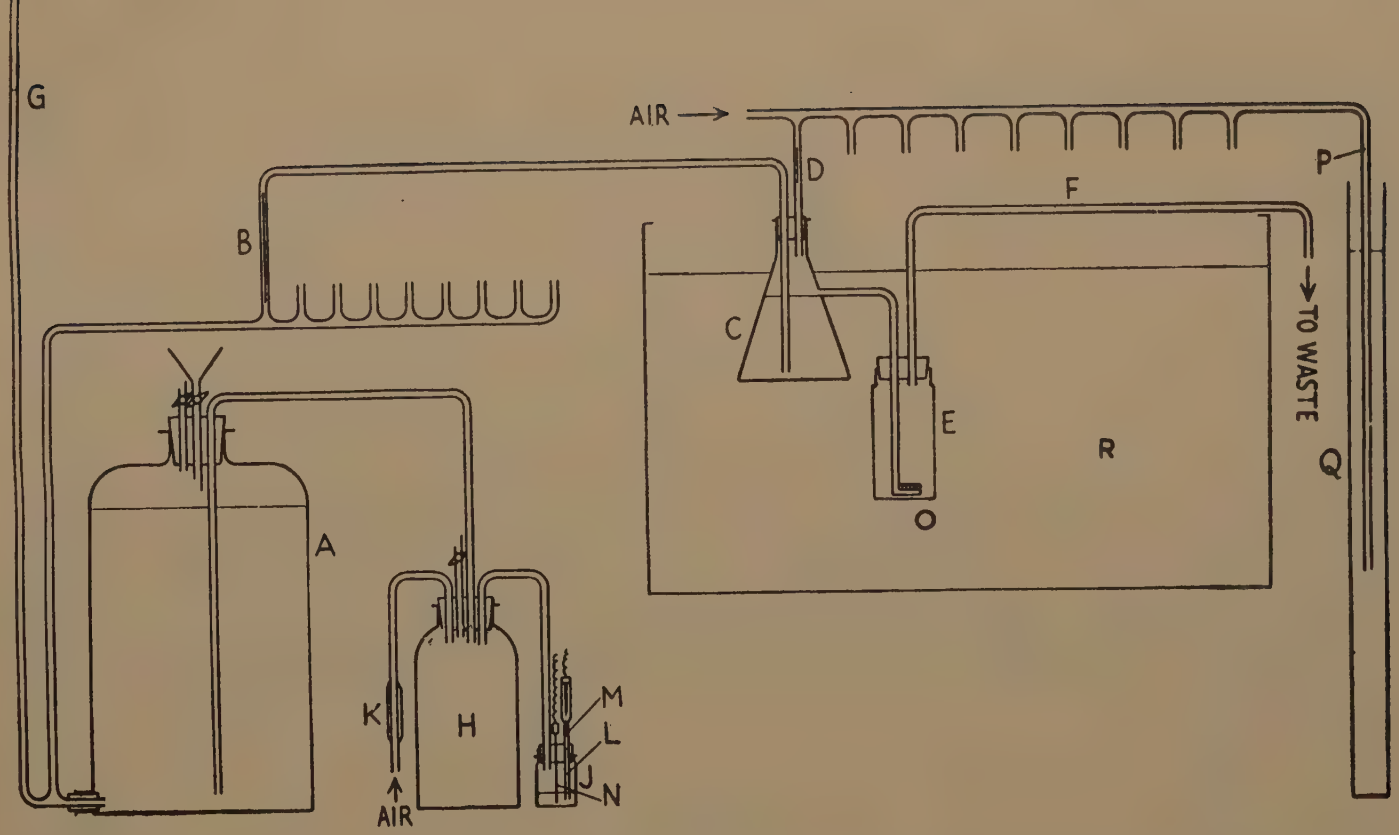


FIGURE 5K

Apparatus for absorption tests from nutrient solutions continuously renewed and aerated under controlled conditions. (*Humphries* 1950).

A = Solution reservoir under pressure.
B = Solution manifold distributor with capillary for uniform distribution to each unit.
C = Temperature equilibration vessel and air intake.
D = Air manifold distributor with capillary resistances for uniform flow of air.
E = Culture or absorption vessel.
F = Outlet for air and waste solution.
G = Manometer to measure " head " of solution.
H = Pressure bottle.
J = Small bottle of mercury.
K = Non-return valve at inlet from air pump.
L = Narrow bore tube.
M = Adjustable metal " make and break " contact with mercury in L.
N = Permanent metal contact in mercury.
(M and N operate a relay to switch off pump when pressure causes mercury to rise in L and complete circuit. Sensitivity $\pm$ 1 cm H_2O).
O = Sintered air and solution distributor at base of culture vessel (porosity O).
P and Q = Adjustable water head for regulating air pressure available to cultures.
R = Thermostat bath.

Eaton (1941 a) also used an airlift to raise the solution above the sand to provide surface application and downward displacement of surplus liquid. The reservoir and culture vessel, shown in Figure 6 B, comprised a standard pair of glazed units. Excess liquid was drained through an aluminium silicate brick of high porosity (approximately 20 ml/min/sq. in.) which was used to support the sand. A layer of gravel was placed between the sand and the porous tile. The airlift was concentric with the overflow and passed up through the middle of the culture vessel. In this respect Eaton's equipment requires more attention and manipulation to set up than that of Chapman and Liebig. The equipment was suitable for plants up to the size of tomato. The sand cultures could be dispensed with and the reservoirs used as water cultures by replacing the upper vessel by a suitable lid, and provision of carbon aerators operated at 5 lb. sq. in. air pressure. The cultures were mounted on revolving tables to equalise environmental conditions and the compressed air lines were connected through mercury seals.

Gauch and Wadleigh (1943) used the method shown in Figure 6 C. The equipment comprised a culture vessel holding 5 times the weight of sand in Eaton's equipment and a reservoir twice as large. The culture vessel was supported on the lid of the reservoir and irrigated from above by an aluminium distributing ring. The use of metal was not perhaps an advantage, and has now been replaced by an easily manipulated thermoplastic tubular material called " Saran " which has also replaced the delivery tubing and T-piece attached to the ring (H. G. Gauch, private communication). The amount of fluid delivered was determined by the size of the delivery bottle and was raised by air pressure. All tubing was blackened to prevent algal growth and the sand was covered by quartz pebbles for the same purpose. Backward flow into the reservoir was prevented by a glass ball check valve. Solenoid valves worked by a time switch controlled the air pressure and release. When pressure was removed the delivery bottle refilled from the reservoir. This use of air pressure provided less effective aeration than the methods already described. The equipment was used to grow tomato, flax, beans, clovers, strawberry and other plants.

Arnon and Grossenbacher (1947) described a sub-irrigation method for large numbers of small pots (Figure 6 D). Solenoid valves were used to control the admission and escape of compressed air used to raise the liquid. Each pot was supplied from a separate reservoir containing 3 L. of solution and connected to the compressed air manifold line. The sand was irrigated for as long as 20 minutes during each hour. The pressure adjustment needed to secure smooth operation was obtained by the inclusion of an adjustable excess-pressure escape. This was made from a long glass tube filled with water to give a " head " of 20 inches equal to that from the surface of the sand to the level of liquid in the reservoir. Thus any excess air pressure above that needed to raise the solution was allowed to escape by bubbling out of the escape valve. Two-gallon sub-irrigated pot gravel cultures were used to grow oats by Watson and Noggle (1947). The pots were irrigated for 20 minutes every 6 hours from 2-gallon reservoirs. Additional irrigations with ferric citrate solution were necessary.

A small manually-operated gravity feed system of sub-irrigation has been described by Paddick (1944). It appears to possess no advantages over other methods of manually-operated sand cultures and its unskilled use might cause waterlogging. Meyer (1945) (1946) used a sub-irrigation technique for pot sand cultures for growing kok saghyz.

AERATION OF CULTURE SOLUTIONS AND STUDY OF ROOT ATMOSPHERE

Importance of Aeration

Aeration is essential for efficient root action, although the needs of different species vary widely. The oxygen tensions in culture solutions may be less than the minimum needed for the normal growth of many plants unless special methods are used.

Lack of aeration was found to cause root rots in citrus by Haas (1932 a) and in sugar cane by Martin (1935) ; it impeded growth or root formation and caused other root infections in apple in studies by Barker (1921) and Pearse (1937) ; and resulted in failure to absorb nutrients by plants grown by Hoagland and Broyer (1936) and Arnon and Hoagland (1940).

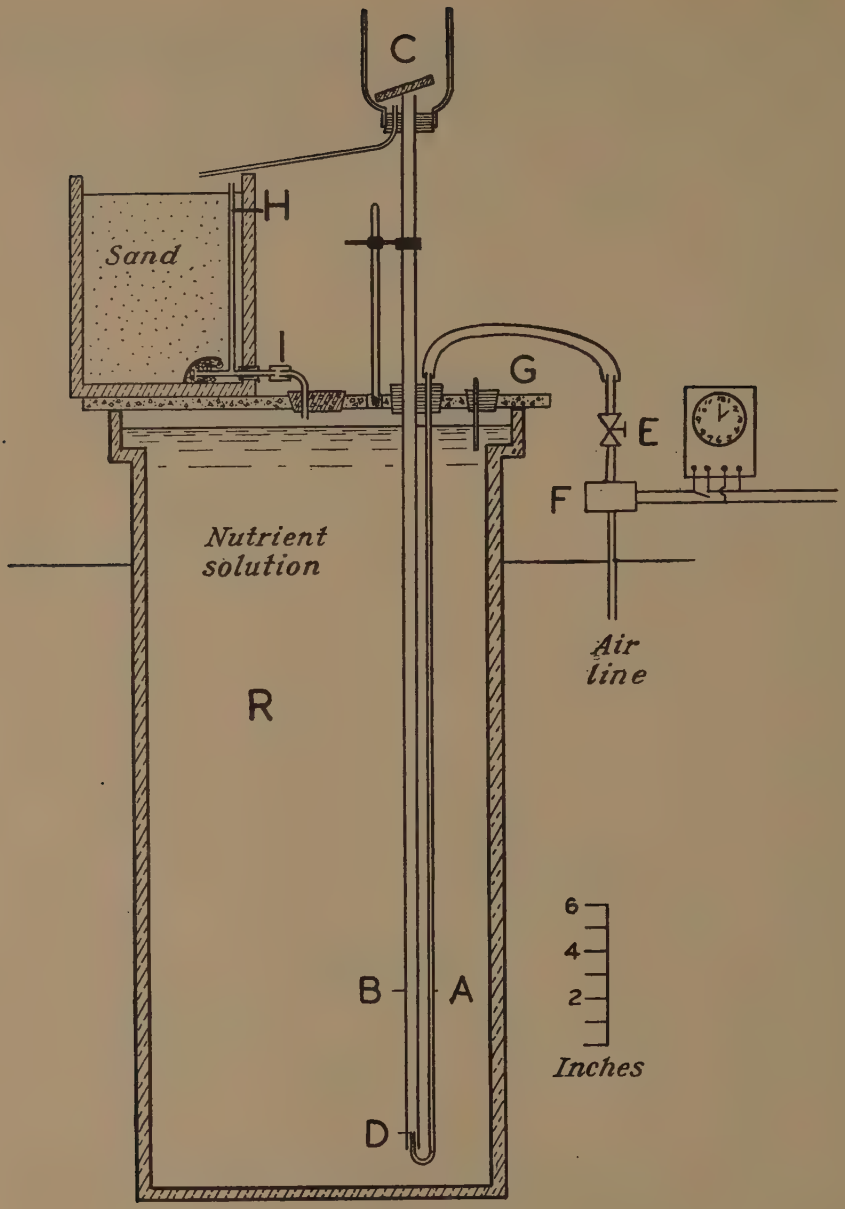


FIGURE 6A

Line drawing showing essential features of automatically-operated sand culture (After Chapman and Liebig, 1938)

A = Air inlet.
B = Ejector member (air inlet device for raising solution to distributor).
C = Distributor with splash baffle.
D = Air outlet.
E = Needle valve.
F = Magnetic valve (solenoid).
G = Volume indicator.
H = Sand culture overflow.
I = Drainage tube to reservoir.
R = Glazed drainpipe solution reservoir.

Arrington and Shive (1936) and Shive (1941) found that uptake of ammonium nitrogen was impaired by poor aeration, but nitrate uptake may even be reduced by forced aeration. Absorption of calcium, potassium and phosphorus was stimulated by increased concentrations of oxygen up to 16 p.p.m. for soya bean and 8 p.p.m. for tomato in experiments by Pepkowitz and Shive (1944). Chang and Loomis (1945) found that accumulation of carbon dioxide in non-aerated culture solutions depressed the absorption of most nutrients and also of water. Gilbert and Shive (1945) found that carbon dioxide accumulation was accentuated by nitrate nitrogen. An additional useful function of forced aeration is that of stirring large volume cultures as illustrated by the work of Sommer (1936), Chapman and Liebig (1940), Arnon and Johnson (1942) and Houghland (1947 (1950), especially where low concentrations or critical pH levels were maintained.

Curtis (1949 b) found that lack of aeration allowed the accumulation of injurious concentrations of nitrite in cultures of citrus and avocado due to denitrifying organisms. Arnon (1937) showed that the need for aeration may depend on the supply of micronutrient elements and the source of nitrogen as already mentioned, and may be less for nitrate than for ammonium nitrogen.

Plants such as tomato appear to have high oxygen requirements of 16 p.p.m. O_2 or more, avocado, citrus, barley and soya bean at 6-8 p.p.m. O_2 are intermediate and rice tolerates low oxygen tensions without apparent disadvantage, according to the work of Durell (1941), Shive (1941), Gilbert and Shive (1942), Vlamis and Davis (1943) (1944) and Curtis (1949 a). The data of Gilbert and Shive (1942) and Pepkowitz and Shive (1944) for optimum oxygen requirements of tomato and soya bean are contradictory. Legumes appeared to require rather more aeration than cereals in the experiments of Allison and Shive (1923) and Piper (1942). The oxygen concentration of unaerated cultures in equilibrium with air is about 2-3 p.p.m. O_2.

Plants injured by excessive aeration include soya bean grown by Shive (1941) and avocado grown by Curtis (1949 a). Gilbert and Shive (1942) found that soya bean showed iron deficiency when over-aerated in the presence of nitrate, but oat appeared to be indifferent to the highest concentration obtainable. Hoagland and Broyer (1936) concluded that over-aeration was as injurious as under-aeration for barley and supplied air at 1·5 cu. ft. per hour for a vessel containing 168 seedling plants. Erickson (1946) found that tomato plants made progressively more root development as the percentage oxygen in the aerating gas was increased until it was equal to that in air. Growth was depressed when 20% or more of oxygen was present. Hopkins *et al.* (1950) found that growth of tomato increased regularly in proportion to the logarithm of the partial pressure of oxygen between 0·5 and 21% oxygen mixed with nitrogen. Root growth of tomato, soya bean and tobacco ceased when oxygen was less than 0·5% in the mixture, but top growth and accumulation of potassium and phosphorus continued slowly. Bond (1950) studied the relation of aeration to growth of soya bean in water culture with nitrate nitrogen and under conditions of nitrogen fixation. There was no consistent benefit due to aeration. Evans (private communication) emphasized the great importance of aeration for cacao, which may otherwise develop iron deficiency.

Methods of Aeration

Freely-drained sand cultures possess the advantage over water cultures of more efficient aeration without the need for special equipment. This is due to the fact that, as fluid gravitates through the sand, air is *aspirated* into the capillary spaces. Undrained sand cultures do not possess this advantage and may easily become waterlogged. The only means of aeration in these latter occurs when the surface sand dries out and air *diffuses* into the spaces.

Water cultures usually need extra attention as regards aeration especially when they are deep, or if for any other reason they present only a small surface area compared with the total volume, or if diffusion of air is restricted by tightly fitting lids.

The realisation of the importance of aeration is not even relatively recent : Andrews and Beal (1919) refer to Sachs as the first to experiment with this problem in 1887, and quantitative experiments were made by Stiles and Jörgensen (1917) with barley, buckwheat and balsam.

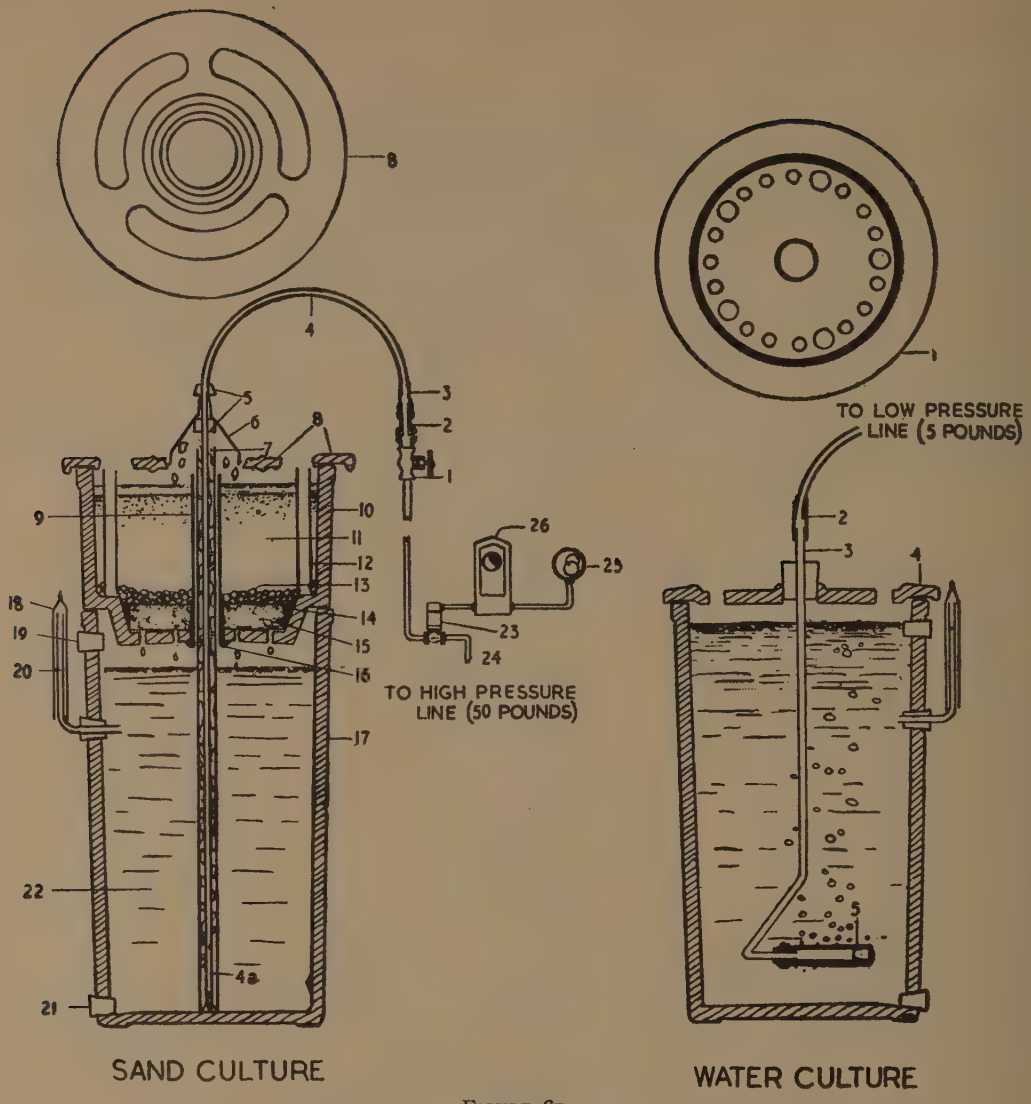


FIGURE 6B

Sand and water cultures (*Eaton*, 1941 a)

Sand culture

1. Air valve.
2. Hose connector.
3. Koroseal tubing.

4 and 4a. Air discharge tube.

5. Funnel supports.
6. Aluminium funnel.
7. Solution delivery tube (air lift principle).
8. Glazed stoneware cover (in section).
9. Glass sleeve and overflow.
10. Sand tray.
11. Quartz sand and magnetite.
12. Air vents.
13. Quartz gravel.
14. Asphalt compound.
15. Porous disc.
16. Red sealing wax.
17. Reservoir.
18. Cap for water gauge.
19. Water addition opening.
20. Water gauge.
21. Outlet for emptying reservoirs.
22. Culture solution.
23. Magnetic air valve.
24. Air pressure line.
25. Time switch.
26. Time switch.

Water culture

1 and 4. Glazed stoneware cover.

2. Rubber or Koroseal connection.
3. Glass or ¼ inch aluminium tubing.
5. Porous carbon aerator.

Efficient aeration may sometimes be achieved without resort to compressed air, if the surface of the solution is extensive and free access to the air is allowed. Haas (1932 a, b) grew citrus in shallow vessels that provided adequate natural aeration in spite of the susceptibility of citrus to root rots, which readily appeared if aeration was restricted by deep vessels or if the roots became coated by precipitated salts. Roots of citrus were injured when the plants were grown in Mason jars without aeration. Hoagland and Arnon (1939) also recommended shallow tanks for large volumes of solution and considered that forced aeration might not be always necessary if a porous bed or other cover that allows free diffusion of air were used, and adequate space left between the cover and the solution surface. Additional forced aeration was regarded, however, as frequently beneficial, and this was clearly confirmed by Arnon and Hoagland (1940) for tomato. Aerated solutions were not, however, superior to sand cultures either for growth or absorption of nutrients. The contribution made by continuous solution-renewal to aeration has already been considered in a discussion of the work of Allison (1921) (1923), Allison and Shive (1923) and others. Where supplementary forced aeration was used by Shive and Robbins (1942) and Clark and Shive (1932) a twofold increase in growth of tomato plants was obtained in spite of the continuously-renewed solutions. Soya bean may also deplete oxygen faster than it is supplied in non-aerated flowing cultures according to Shive (1923). The duration of forced aeration need not be continuous and aeration for 10 minutes of each hour or 4-6 times daily for 30 minutes at a time may be quite adequate under normal conditions where high temperatures are avoided. Continuous aeration, however, is often used, as for example by Chapman and Parker (1942) for citrus, Tanada (1946) for coffee, and Houghland (1950) for potato.

Small-capacity water cultures may not need forced aeration. Brenchley (private communication) found no significant benefit from extra aeration for ·5-1 L. cultures. An indication of the relation of capacity to the need for extra aeration is gained from Table 2. Mulder (1948 a) dispensed with forced aeration in molybdenum-deficiency studies to avoid possible entrainment of impurities in the dust. The elimination of this source of dust from micronutrient-deficiency cultures is easily effected by scrubbing and filtering through water or acid and then cotton wool, as recommended by Lehr (1940), Smith and Bayliss (1942), Wade (1944), Millikan (private communication) and Piper (private communication). Pearse (1937) used charcoal to remove oil spray from a compressor.

Several methods have been described to achieve effective aeration. Early arrangements were those of Weatherwax (1914) and Andrews (1919). These were based on the entrainment of air in a stream of water. A water suction pump also can be made to provide a supply of air under pressure by arranging that the pump discharges into a reservoir so that air can escape only slowly through a capillary whilst the water is slightly impeded in flowing to waste. A water seal is thus created and air pressure builds up in the reservoir. This method has the disadvantage that, should the air-escape resistance slacken due to leakage, the reservoir and air lines rapidly fill with water. A safer arrangement providing less pressure is to discharge the pump into the top of a tall column or wide tube placed with its bottom open and inside another wider and slightly shorter tube open at the top and closed at the bottom. The air is led out from the top of the inner tube and the pressure is equal to the head of water displaced by the inflowing air. Franco and Costa (1949) described a simple device to supply intermittent aeration automatically from a mains water supply. Air provided in this manner is practically dust-free and not contaminated by oil, as when a compressor is used. Spray is almost certainly entrained and must be filtered out for precise work. Entrainment of air in a moving column of fluid was used by Pearse (1937) (Fig. 6 E), and in the irrigated sand cultures of Chapman and Liebig (1938) and Eaton (1941 a).

The actual means of admitting air to the culture solution varies. The simplest is a 0·5 mm. capillary tube with the end ground to 45°, used by Lehr (1940) and Shive and Robbins (1942). Piper (private communication) and Evans and Maskell (private communication) (Fig. 5 J) used capillaries to provide fine bubbles and sufficient resistance to ensure even distribution from a manifold to several cultures at once. Street *et al.* (1946) used small, finely perforated bulbs to admit fine streams of air bubbles. The air was cooled before compression

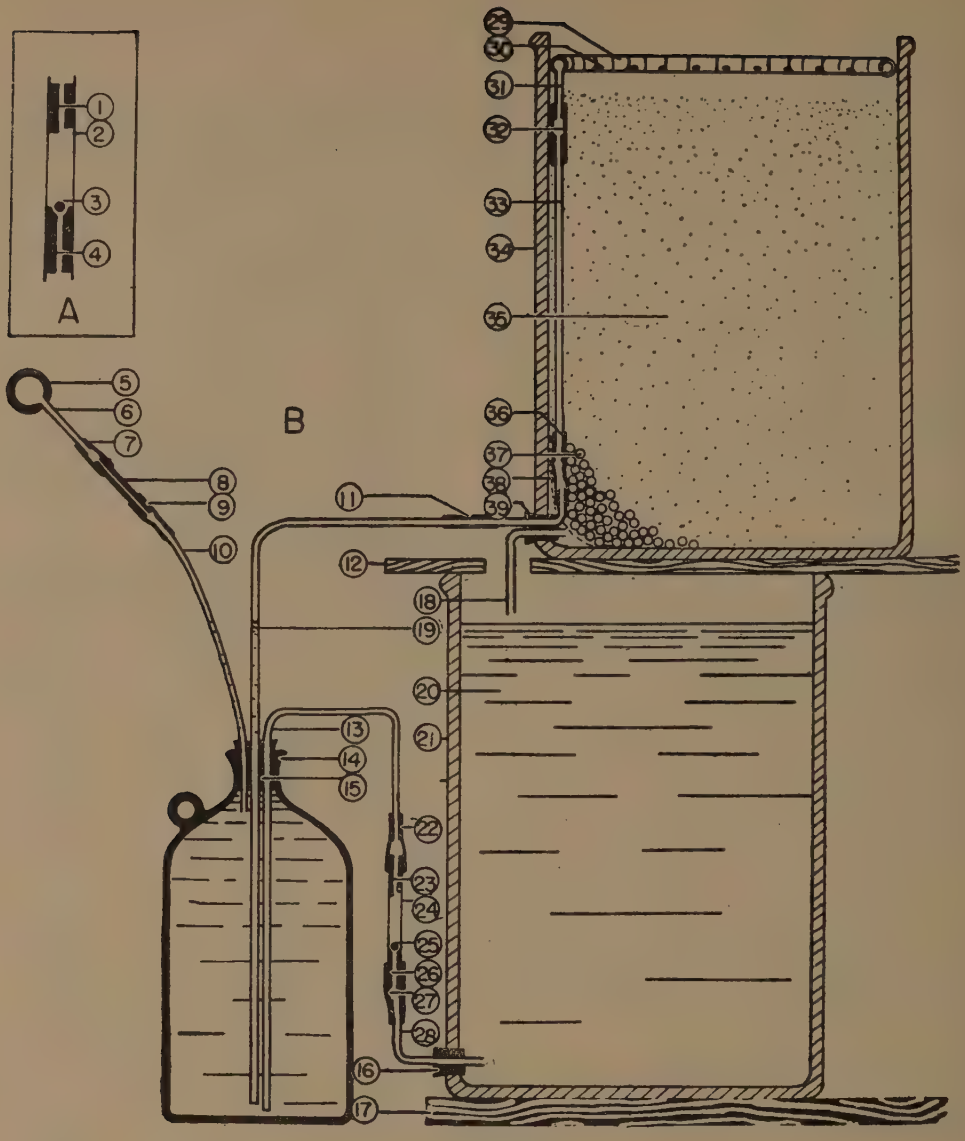


FIGURE 6C

Cross-section view of sand culture apparatus (*Gauch and Wadleigh*, 1943)

A. *Glass bead check valve.*
1. Rubber tubing sealed to the glass tube.
2. 10 mm. O.D. glass tubing 3½″ in length.
3. Glass bead 4 mm. in diameter (prevents flow solution into reservoir when air pressure forces solution from delivery bottle up to sand culture but permits refilling of delivery bottle when air pressure is released.
4. Rubber tubing sealed to the glass tubing (2).

B. *Sand culture apparatus.*
5. ⅜″ galvanized iron pipe serving as air-supply line for each group of 10 cultures.
6. ⅛″ copper tubing outlet from main air-line pipe.
7, 9. 11, 22, 27, 32, 36. Rubber tubing connections.
8. 2″ piece of ½ mm. capillary tubing.
10. 3 mm. O.D. glass tubing.
12. Wooden plank with holes 20″ apart.
13, 18, 19, 33. 5 mm. O.D. glass tubing.
14. ½ gall. glass jug.
15. No. 6 Long-taper rubber stopper.
16, 39. No. 3 rubber stopper.
17. Wooden skid.
20. Culture solution.
21, 34. 5-gall. glazed crock.
23, 24, 25, 26. A—1, —2, —3, —4 respectively.
29. Soft aluminium or plastic tubing (3/16″).
30. 1/32″ outlet hole in tube.
31. Glass T-tube.
35. Fine quartz sand.
37. Rock quartz.
38. Wire screen or gauze 2″ × 2″.

and afterwards bubbled through water to saturate it with water vapour before storage in a reservoir used to supply the cultures. Furnstall and Johnson (1936) recommended pyrex glass sintered filters as the most satisfactory means of aeration of solutions. The air stream is broken up into minute bubbles of large aggregate surface area and the aerators can be cleaned for use in micronutrient techniques. Carroll (1939) described a procedure for making such sintered aerators, which are also available commercially. Pyrex glass sinters have been used by numerous workers for highly refined water culture experiments including Hoagland and Broyer (1936), Liebig *et al.* (1942), Barker and Broyer (1942), Liebig *et al.* (1943 b) and Vanselow and Datta (1949). Porous carbon tubes were used by Arnon (1937), Arnon and Johnson (1942), Arnon *et al.* (1942), Eaton (1941 a) and Ramig and Vandecaveye (1950) to aerate large culture tanks, and were supplied with air at 5-10 lb. sq. in. The use of rubber tubing for connections may require care, since rubber tubing is likely to contain zinc, according to Furnstall and Johnson (1936). Piper (private communication) recommended a flexible plastic tube composed of " Tygon," used also by Ramig and Vandecaveye (1950). Polythene tubing might also be satisfactory. A wide range of oxygen tension can be obtained by use of oxygen mixed with different proportions of nitrogen from cylinders. Hoagland and Broyer (1936) found, however, that the minimum O_2 tension produced with nitrogen only was 0·05 p.p.m. (determined by Winkler's method) due to atmospheric leakage through the cotton wool supporting the plants. Hopkins *et al.* (1950) also used mixtures of oxygen and nitrogen. An ingenious device controlled the oxygen proportion within $\pm$ ·05%.

Sealed Cultures for Study of Root Atmosphere

Methods for the study of effects of controlled oxygen tensions on plant growth in sealed water cultures have been described by Shive (1941), Gilbert and Shive (1942) and Pepkowitz and Shive (1944). Sealed cultures have also been devised to study the atmosphere in contact with the roots of culture plants. Where sand cultures are used, the seal over the surface and around the plants may be effected by applying a wax to the surface of the sand. This procedure was adopted by McCall (1916 a), Shive (1918 a), Wolkoff (1918), Johnston (1920) (1925) and Bakke and Erdman (1923) to reduce water loss and to prevent salt accumulation at the surface of the sand in undrained containers. The wax used was made according to the formula of Briggs and Shantz (1911) (1912) and had a melting point below 40° C. It consisted of 80% paraffin wax (Mpt 45° C.) and 20% petrolatum. This composition softens in the sun and a " warm weather mixture " was composed of beeswax and 10-30% beef tallow or 8-12% petrolatum. Beeswax alone shrinks and cracks. The use of sealed cultures merely to reduce water loss is not justified owing to the impaired aeration that results.

A more elaborate arrangement was used by White and Childers (1944) to study root respiration and atmosphere in sand culture. The container lid was sealed by grafting wax round the rim and round the actual hole surrounding a tree or other single-stem type of plant. A thermograph was also sealed inside the container. The root atmosphere was displaced upwards by pumping solution under slight pressure into the sand container through a basal aperture. The displaced gas was collected through capillary bore tubing in a pneumatic gas holder over water and provided with a sampling point. By closing and opening the appropriate stopcocks the gas holder automatically sank and emptied in readiness for the next cycle. Fresh air or other gas was drawn into the sand culture by allowing the culture solution to return by gravity to the reservoir. The culture solution had a pH above 5·0 to prevent absorption of CO_2 and contained 5 p.p.m. of copper to inhibit the respiration of microorganisms. This high concentration was not apparently injurious to tree roots. Sealed cultures were also used earlier by Dustman (1925) to study the evolution of carbon dioxide by roots of culture plants. The culture vessels were sealed with a mixture of moistened clay and vaseline, and air was drawn through by aspirators.

Gregory and Woodford (1939) described an ingenious technique for the study of the uptake of salts and respiration of various zones of the roots of culture plants. The root was made to penetrate a number of thin perforated sheet rubber discs which comprised the diaphragms between small cells ranged along the length of the root. Each cell had a two-way lateral

aperture for control and sampling of the root environment. Broad bean was considered a suitable experimental plant. The apparatus was suited to the measurement of gaseous exchange or nutrient uptake from solutions allowed to flow through the cells, or to the measurement of water uptake by attachment of a micropotometer. Earlier methods intended for similar purposes were also reviewed. A sealed water culture technique used by Woodford and Gregory (1948) is described in the Section on absorption of nutrients from solutions.

Jones *et al.* (1949) used a layer of colourless russian oil to seal the surface of water cultures. They found that oat plants were unable to assimilate nitrate, and nitrite accumulated, unless either oxygen or manganese was present. The limiting oxygen tension at equilibrium under the oil layer was 0·5 p.p.m. with plants and 2 p.p.m. without them.

THE STUDY OF FACTORS AFFECTING THE ABSORPTION OF NUTRIENTS FROM CULTURE MEDIA

The rate and amount of absorption of a particular nutrient by plants depends on a number of variables, including pH, aeration, source of nutrient or age of plant, and some of these factors have been discussed in other sections. This account is intended only to draw attention to some of the experiments that have been devised to test the absorption of nutrients under different circumstances.

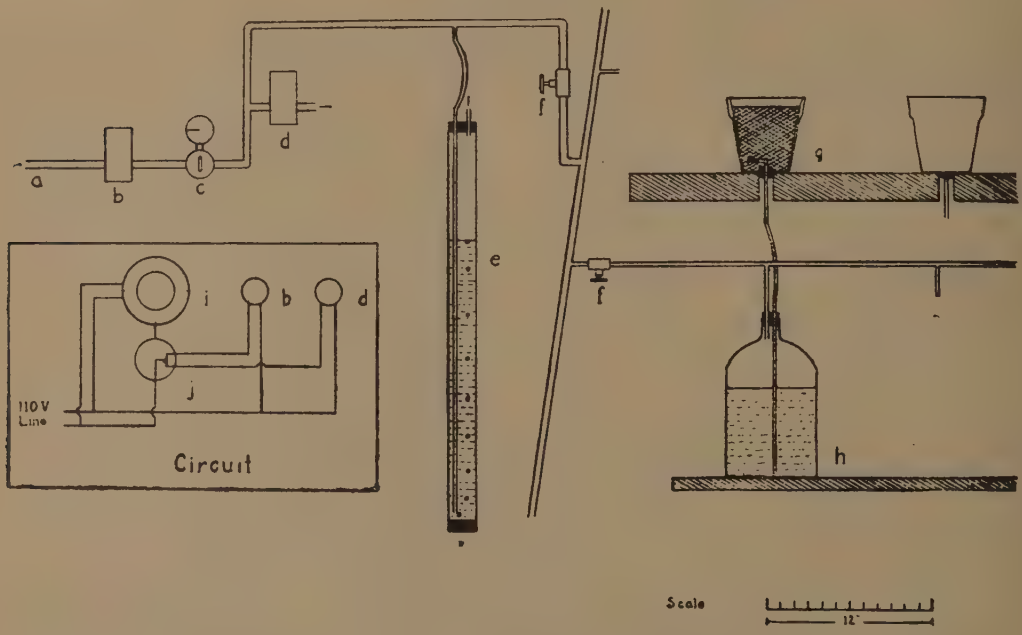


FIGURE 61

Automatic apparatus for flooding numerous pot aggregate cultures (*Arnon and Grossenbacher*, 1947)

a. Compressed air line.
b. Solenoid valve-intake.
c. Pressure regulator.
d. Solenoid valve-exhaust.
e. Adjustable escape valve.
f. Shut-off valves.
g. Pot with aggregate.
h. Solution reservoir.
i. Electric clock.
j. Timing switch.

The effects of concentration of nutrients on absorption were studied by Hoagland (1919) and extended by Arnon and Hoagland (1940), who devised a nutrient solution intended to represent the proportions of various ions absorbed by the plant.

The effect of plant age on absorption was studied by Breazeale (1923), Jones and Skinner (1927), Brenchley (1929), Naftel (1931) and Stahl and Shive (1933). The usual procedure was either to grow plants sown at the same time and harvest them at intervals (Breazeale, Brenchley), or to transfer them at various stages of development (every 1-3 weeks) for a short period (24-48 hours) to a limited volume of solution (400-1,000 ml.) of known composition and to determine the amount of nutrient depletion (Jones and Skinner, Naftel, Stahl and Shive). Stahl and Shive (1933) used a careful technique to wash the roots, and transfer the plants to the absorption test vessels. Plants were withdrawn in small batches for analysis every 4 hours over a period of 48 hours and the test was repeated every 12 days on a fresh batch sown at the same time as all the others.

Breazeale (1923) tested also the effect of duration of absorption period on uptake and found that whereas uptake of nitrogen and potassium was constant for a 24-hour period, uptake of phosphorus declined after 12 hours. Brenchley (1929) found that nearly all the phosphorus was taken up by barley before ear formation.

Techniques for the study of absorption from large volumes of dilute solutions were described by Parker (1927) and Parker and Pierre (1928). The rate of absorption of phosphorus in these experiments was determined by analysis of the solutions twice daily. Arrington and Shive (1936) also analysed the residual solution in tests designed to study effects of pH and aeration on absorption of nitrate and ammonium nitrogen. They used a careful technique in which a relatively large volume of flowing culture solution was allowed to come into contact with the roots of the test plants and was then retained for analysis. The plants had already been grown under similar conditions. Non-aeration resulted in reduced uptake of both sources of nitrogen.

Arnon *et al.* (1942) also examined the effects of pH on absorption by growing plants (already of " low salt " status and conditioned to the type of nutrient to be used), for short periods in large containers, with culture solutions aerated (stirred) and accurately maintained at different pH levels. The absorption of nutrients was determined by analyses of the culture solutions. Low pH values impaired uptake of calcium, phosphorus and magnesium. Arnon and Hoagland (1940) also used solution analysis in their attempts to devise a solution to parallel the absorption by the plant. The importance of the " conditioning treatment " before the actual test was emphasised by Arnon *et al.* as a means of lessening shock due to the new conditions.

The frequent use of solution, rather than plant analysis, to determine absorption merits comment. The method is generally preferable because it does not entail analysis of the *whole* of *all* the culture plants, which is the only satisfactory alternative to determine nutrient uptake. This is because each part of the plant may contain different concentrations of the elements and different individuals may also vary. Analysis of the nutrient solution may also eliminate the need to ash or digest the material before analysis. On the other hand greater concentrations of the nutrients will be found in the plant than in the solution, and if the latter are of suitable physiological concentration and of sufficient volume for the number of plants used and the period of the experiment, the changes will be small, as in fact is always implied in the handling of a properly conducted culture experiment. Errors will therefore be relatively large and considerable care is necessary to ensure that any precipitated material is accounted for in the balance sheet.

The work of Hoagland and Broyer (1936) established many of the conditions conducive to salt accumulation by roots. Excised roots of barley were used as the main experimental material. Seedlings were raised in large numbers by a reproducible germination technique. The plants were transferred when 7 days old to a dilute nutrient solution for 2-3 weeks. They were not aerated during this period but sufficient aeration was obtained by the use of shallow 4-L. tanks with loose covers. One hundred and sixty-eight plants selected for uniformity were used in each experiment. The nutrient solution was unchanged during the 2-3 weeks'

period. This resulted in a " low-salt " status in the roots conducive to rapid accumulation, but not so deficient as to cause injury or impairment of capacity for growth. At this stage the roots were excised whole just below the seed level, washed, centrifuged slowly in cloth bags suspended in place of the usual buckets, and transferred to 4-L. glass jars or 4-L. shallow dishes for the absorption tests. Aeration was provided with sintered aerators and air was passed at 1·5 cu. ft./hour per batch of 168 roots. The course of absorption was followed by washing, centrifuging the roots (which consistently removed 99% of the surplus moisture) and then analysing the sap expressed from frozen roots. Occasionally the residual solutions were also analysed.

The results obtained present important points for consideration in relation to the problems of nutrient uptake by plants. Salt accumulation was most active in young tissues that possessed the capacity for active growth. When more than 6 weeks old, barley roots had a greatly reduced capacity for accumulation. Aeration was particularly important for rapid accumulation. The salt status had a profound influence on accumulation and a " low salt " status was especially conducive to rapid salt uptake. This was depressed if carbohydrate reserves were depleted. The period during which rapid accumulation occurred was limited. Uptake was rapid for 12-19 hours and " tailed off " after 24 hours and was often nil or negative between 30 and 50 hours after commencement of the test. Evidently the absorption period in experiments of this nature should be limited to 12-24 hours' duration.

Woodford and Gregory (1948) devised a simple and adaptable technique to study accurately the uptake of nutrients and their interrelationships with root aeration and respiration under sterile conditions. The apparatus comprised a special culture vessel sealed with a bakelite lid. This was designed to hold 54 barley seedlings about 7-10 days old which were sealed into the lid with wax. The culture vessel had a capacity of 100 ml. Nitrogen, mixed with oxygen as required, was bubbled upwards through a sintered glass disc sealed across the base of the culture vessel and the flow of gas prevented loss of solution here. The nutrient solution was circulated automatically by pressure from a cylinder of nitrogen gas. Circulation was achieved by the inclusion of a simple non-return glass poppet valve and by arranging that the incoming solution flowed down to the culture vessel after elevation into a small vessel arranged so that the outlet was at the bottom whilst the inlet was raised. This vessel was at atmospheric pressure and the nitrogen gas escaped from a tube led out at the top and through the carbon dioxide absorption coils. After leaving the culture vessel the returning culture fluid was boiled in a small electrically-heated boiling tube and the expelled gases were led through a condenser to the carbon dioxide absorption apparatus. The boiled solution was automatically siphoned out and led through a constant temperature cooling coil and the poppet valve back to the chamber, where the nitrogen gas pressure was applied, and thence recirculated. A thermometer was fitted into the solution inlet and a third tube at the top of the culture vessel was attached to a filling funnel and closed by a stopcock.

The seedlings were raised by a technique similar to that of Hoagland and Broyer (1936), but the temperatures during light and dark periods in the growth chamber were maintained at 22° C. and 18° C. respectively. After removal of pericarp and endosperm the seedlings were sealed into the holes in the bakelite cover by wax made of vaseline and beeswax melted at 35° C. The bakelite lid was sealed on to the culture vessel with sodium cerate and beeswax and when complete the surface was further sealed by pouring 1·5% agar solution at 35° C. into a mould formed by a half-inch deep metal ring placed around the plants. Before finally sealing the apparatus the seedlings were conditioned to the nutrient conditions by growing them in diluted nutrient solution for 3 days in the growth chamber.

The course of nutrient absorption was followed by analysis of the culture solution before and after a 5-hour period. The respiration of the roots was measured by absorption and determination of carbon dioxide entrained in the aerating gas mixture and expelled from the boiling culture solution after it left the culture vessel. The nutrient solution was relatively concentrated but contained no molybdenum in the micronutrient supplement. The technique was more elaborate than that of Hoagland and Broyer (1936), but was more accurately

controlled and permitted measurement of respiration and, if required, use of sterile culture conditions.

Humphries (1950) also devised a simple apparatus for the study of absorption of excised root systems. The nutrient solution was continually renewed and aerated. Several absorption chambers could be maintained simultaneously at a controlled temperature (Fig. 5 K).

Where it is required to study the *availability* of nutrients presented in the absorbed state as in the experiment of Horner (1936) or Arnon and Meagher (1947), analysis of the foliage may be a convenient and satisfactory procedure. The conclusions in these instances are based on the comparative data obtained from analyses of carefully selected comparable material and on the total yields of dry weight of the plants. Details of the most suitable organs and sampling techniques to use may be obtained from a critical review of the relation of chemical composition to nutrient status by Goodall and Gregory (1947). Fully-expanded but not senescent foliage is often a satisfactory region to indicate nutritional status.

TREE CULTURES AND LARGE-SCALE TECHNIQUES

Tree Cultures

Several species of trees have been grown successfully in sand or water cultures and there do not appear to be many problems peculiar to this aspect of culture experiments, though it should be remembered that trees are perennials and that larger containers may be required than for vegetables. Early records of tree cultures go back to those of Duhamel du Monceau (1758) who grew oak, walnut, chestnut and almond trees in water cultures for periods of from 3 to 8 years. Small containers were preferred, possibly because of better aeration. Trees may grow normally for considerable periods in culture experiments. Pearse (1940) reviewed early work and referred to Wolfe and Knop who grew oak trees in water culture for 15 years. The largest recorded experiment, however, is probably that of Lagasse (1929) who maintained peach trees in sand in 12-foot diameter concrete pits (holding over 22 tons of sand) for more than 19 years in a healthy condition. Chapman (private communication) records that citrus trees have been maintained in vigorous growth for 16 years in large-scale water cultures, described below, without any signs of deterioration.

Numerous experiments have been made with apple trees, mainly in sand culture, by Wallace (1921) (1924) (1930), Davis (1930), Tiedjens and Blake (1932), Nightingale (1934), Nightingale and Blake (1934 a), Blake *et al.* (1937), Batjer and Degman (1940), Hill and Johnston (1940), Waltman (1940), Cullinan and Batjer (1943), Read (private communication), and in water culture by Barker (1922) and by Pearse (1937) (1940), both of whom found that spraying or sprinkling the roots of trees suspended in a humid atmosphere was the most satisfactory procedure for water culture (Fig. 6 E). Rootstocks used have included "free" stocks, "Paradise" stocks and Malling rootstocks especially Nos. I, II, V, IX, XII and XVI. In most of the sand culture experiments the trees were 1-3 years old, heavily root-pruned to remove fibrous roots before planting, and stems were cut back to 10-20 inches above the sand, or even to only 2 buds as in the work of Batjer and Degman (1940). The nutrient requirements are fairly normal and levels of nitrogen and potassium up to 8-12 m.eq./L. and 3 m.eq./L. respectively appear satisfactory, whilst only 0·3-1 m.eq./L. phosphate is usually adequate.

Read (private communication) found it necessary to reduce somewhat the potassium (and nitrogen) levels given to apple trees in sand culture in Victoria, Australia, owing to the incidence of magnesium deficiency under conditions of higher light intensity. Wallace (1943) has described how apple trees grown in sand cultures in the open showed nitrogen deficiency which was not apparent under glass. The need for regular leaching has already been stated.

Peach trees have also been extensively grown in sand cultures. The long-duration experiments of Lagasse (1929) indicate the adaptability of the peach to sand-culture studies. More recent experiments include those of Nightingale and Blake (1934 b), Weinberger and Cullinan (1936), Davidson and Blake (1937), Cullinan *et al.* (1938), Waltman (1940), Hayward and Long (1941 b), Cullinan and Batjer (1943), Brown (1945), and Hayward *et al.* (1946).

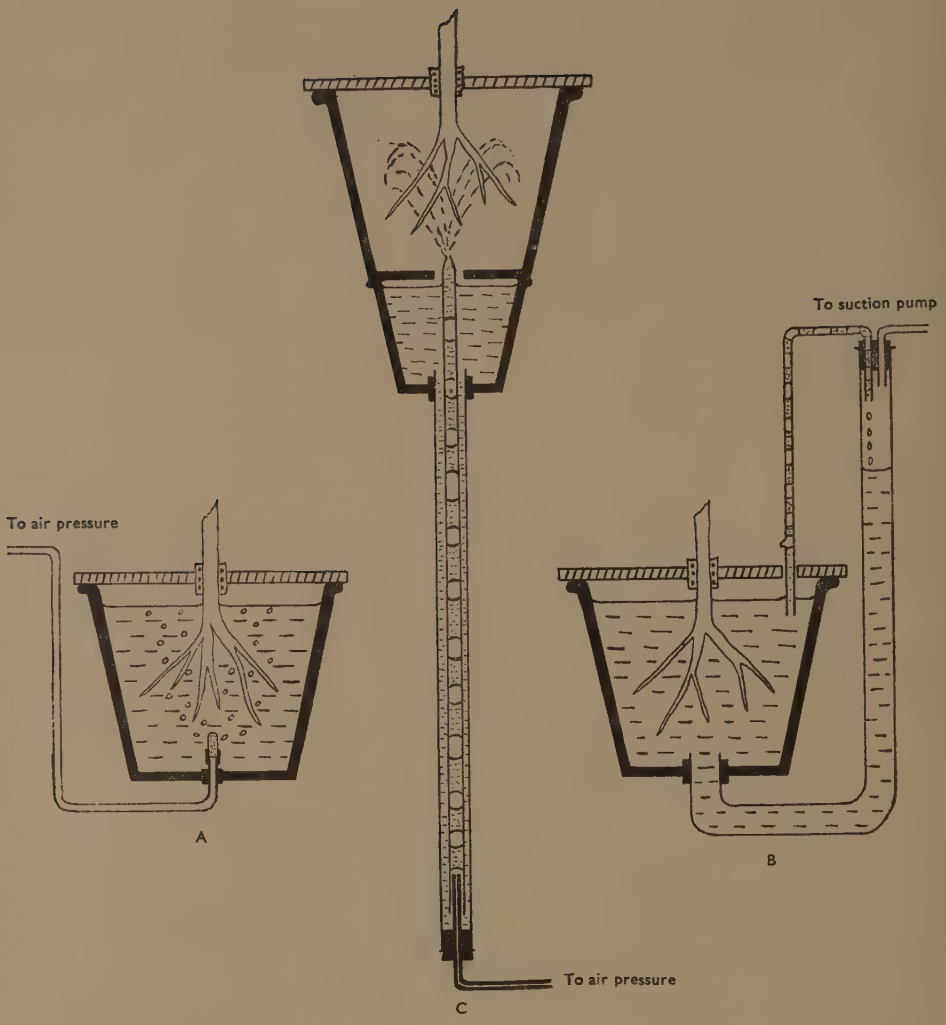


FIGURE 6E

Methods for growing apple trees in aerated water cultures (*Pearse*, 1937)

A = The bubbling method.

The trees were grown in 12-inch plant pots which had been coated with paraffin wax. Their roots were immersed in the solution, aeration being effected by glass tubes leading to the bottom centre of the pots. The air stream was broken up into fine bubbles by means of a small Alundun Thimble R.A. 98. Each pot contained 8 litres of solution.

B = The circulating method.

Similar to A except that aeration was effected by removing the solution from the top of the pot, aerating it, and then re-feeding it into the bottom of the pot. The solution was kept constantly circulating by means of a suction pump.

C = The spray method.

The roots were suspended in the pot and were constantly sprayed with the nutrient solution.

As with apple trees, severe root pruning, cutting back and cold storage (38° C.) prior to planting have all been commonly practised. Nightingale and Blake (1934 b) grew 5 maiden trees in each 10-inch pot. Potassium requirements may be less than for apples and phosphorus requirements are also low, 0·4 m.eq./L. PO_4''' being apparently adequate; Cullinan *et al.* (1938) found that iron deficiency may occur if potassium is at all deficient. 0·5 m.eq./L. calcium may be adequate, especially in large irrigated sand beds such as those used by Hayward *et al.* (1946).

Some experimenters, e.g. Nightingale (1934), Blake *et al.* (1937), kept trees in cold storage at 35-40° F. or 40-45° F. until they were planted under glass in March; Nightingale and Blake (1934 b) gave peach trees similar treatment at 38° F. An adequate dormant period is important. Suitable containers have been discussed and noted in Tables 1 and 2. Continuous renewal of solution even at high rates of 36 L./hours appears to be tolerated by trees in sand cultures according to Nightingale (1934).

Citrus species have been grown in numerous sand and water culture experiments including (hose by Reed and Haas (1923 a, b) (1924 a), Haas (1930) (1932 a, b) (1937) (1940) (1946) t1949), Haas and Reed (1927), Haas and Klotz (1931), Chapman (1939 and unpublished), Chapman and Liebig (1937) (1938) (1940), Chapman and Parker (1942), Chapman *et al.* (1937) (1939) (1943) (1947), Liebig *et al.* (1942) (1943 a, b), Guest (1944), Guest and Chapman (1944), Wade (1944) and Roy and Gardner (1946). In the experiments of Chapman (unpublished) and Chapman *et al.* (1943) (1947) trees that had come into bearing for several years were used. Adequate aeration is especially important with citrus for root development in water culture and iron requirements are high. Stocks may be raised either as seedlings or cuttings. *Citrus* species are useful indicators for zinc deficiency and deficiency or excess of boron.

Other trees grown in culture experiments include French prune selected by Colby (1933) and Myrobalan plum used by Hoagland (1941) and grown in aerated water cultures. *Rhododendron* was grown in sand cultures with continuous solution-renewal by Spencer and Shive (1933) and various species of coniferous trees were grown in sand cultures by Addoms (1937) and Mitchell (1939) and in water culture by Smith and Bayliss (1942) and Smith (1943). Mycorrhizal association may be poor under these conditions according to Addoms (1937). Tea bushes were grown in sand cultures holding 42 kg. by de Haan and Schoorel (1940) and de Haan (1941). Details of sand and arrangement of nutrient reservoirs were given and also references to earlier work with this crop. Gadd (1928) grew tea in water cultures. Work on coffee in water culture was reported by s' Jacob (1938) (1939), van der Veen (1940), Mayne (1940) and Tanada (1946). Alben *et al.* (1942) grew pecan in sand cultures. Greenwood and Posnette (1947) at Tafo, West Africa, and Maskell and Evans (1951, private communication) at Trinidad, grew cacao in water and sand cultures respectively. Seedling plants bearing only a few leaves were used. These were raised either by sowing seed or by rooting leaf cuttings, with the aid of a rooting hormone. The water cultures were 800 ml. capacity, darkened jars and were aerated daily. The nutrient solution was based on that of Reeve and Shive (1944) with 50 p.p.m. K, and 0·5 p.p.m. B and Zn, 1 p.p.m. Fe and 0·25 p.p.m. Mn. Deficiency effects of all macronutrients, boron, iron and manganese were readily produced, and responses obtained also to application of copper, zinc or molybdenum. Wallace (1932) reviewed the general problems of the nutrition of trees, especially fruit. Nutrient requirements and effects of rootstock and variety were described.

Large-scale Installations

No precise definition of the term " large-scale " is necessary, but for purposes of convenience it is intended to include either large individual cultures, each holding several gallons of solution or hundreds of pounds of sand or installations and techniques devised for the manipulation of large numbers (about 1,000 or over) of separate but not always large cultures. Hydroponic and commercialised installations are mainly excluded from this discussion. Details of such installations are available in publications by Biekart and Connors (1935), Withrow and Biebel (1936), Laurie and Kiplinger (1940), Stoughton (1941) (1947), Davidson (1946)

Miles (1947) and Withrow and Withrow (1948). Many points applicable to large-scale technique have already been described in the Sections on nutrient application and operation of sand or water cultures. Large units may be useful either to grow trees for prolonged periods or to maintain pH values or low nutrient concentrations between narrow limits or to grow large numbers of plants under identical conditions. The size of the units, however, usually limits the number available and may thus impair statistical treatment of the data obtained by their use.

Water cultures appear to be less frequently adapted to large-scale operation than sand cultures, but Arnon and Hoagland (1940), in a study of culture techniques, pointed out that " no evidence was found in support of the view that large-scale water-culture operations rest on new principles unknown to the usual small-scale scientific experimentation." Frequent periodical additions of an iron compound are usually necessary to prevent chlorosis, and control of iron nutrition is difficult under these conditions. Large-scale water cultures may require more attention to aeration provided either from aerators or naturally, and Arnon and Hoagland (1940) recommended that the depth of the tanks should be limited to about 8 inches. When natural aeration is used the surface of the solution should be 2-3 inches below the covers and provision for free access of air should be provided. Forced aeration was found by Arnon and Hoagland (1940) to give improved yields of tomatoes over non-aerated cultures in spite of the shallow dimensions in relation to the large surface area. The shallow tanks used by Gericke and Tavernetti (1936), Arnon and Hoagland (1940) and Arnon and Grossenbacher (1947) were 10 feet long by 2·5 feet wide by 8 inches deep and held 460 L. solution and 20 plants each. Deeper tanks holding 1,000 L. were used by Tidmore (1930) and Sommer (1936), who also supplied forced aeration.

Davidson (1946) designed a simple tank for large-scale water-culture work. The solution was continuously circulated by a small centrifugal pump and the overflow was returned to the reservoirs as a spray which was intended to provide additional aeration. Solution heating was not recommended, in agreement with conclusions of Arnon and Hoagland (1940) and contrary to those of Gericke and Tavernetti (1936).

Chapman (private communication) has given details of the large-scale water cultures used to grow citrus trees at Riverside. The containers are sections of concrete conduit 3 feet in diameter and 4 feet long. These are sunk in the ground to a depth of about 2 feet 9 inches, and the bottom is made by pouring in concrete. The surface is treated with an asphalt-water emulsion resembling " tree-seal." This material resists breakdown and adheres well to the concrete. The trees are transferred to fresh containers when necessary. Lids are made in 2 semicircular parts with a central hole. The trees are supported by wires suspended from a framework of pipes. These carry the air to the culture aerators. The containers hold 700 L. of solution. This is aerated continuously and changed about once a month. The trees often exceed 7 feet in height, and grow vigorously, but chlorosis readily appears unless iron is added several times weekly. The solution is preferably maintained at pH 4, to improve iron availability and to avoid attacks by root fungi which occur at neutral or alkaline pH values.

Most large-scale equipment has been designed for sand-culture work. The sand beds used by Eaton (1931) were shallow, bitumen-painted iron tanks, 12 feet long by 1·5 feet wide by 11 inches deep and had V-section bottoms to facilitate drainage. The V was filled with gravel overlying a 50-mesh gauze placed over the drainage tube along the centre of the V. Sand (40-mesh) occupied the rest of the space. The beds were flooded from the surface by allowing solution to flow from 50-gallon barrels suspended above the beds. The barrels were refilled by suction pump from reservoirs below the level of the sand. An occasional sub-irrigation was used to prevent compaction of the sand bed.

The shallow beds for annual crops used by Eaton (1936) were somewhat larger and measured 15 feet long by 2·5 feet wide by 13·5 inches deep. The reservoirs held 90 gallons, but were later increased to 550 gallons, Eaton (1941 a) (1942 b), and the pumping motor was placed below the upper level of the solution and was thus always primed. Drainage from the sand beds was through a special grade of aluminium silicate brick which replaced the gravel formerly used. These beds retained 70 gallons (300 L.) capillary moisture in the sand

after drainage (Fig. 6 F). The ratio of reservoir capacity to sand surface area was thus greater than allowed for by Davidson (1946) (below). Eaton (1942 b) pointed out that upward flooding was inferior to downward displacement of nutrient solution.

Sand beds used by Arnon and Hoagland (1940) were 10 feet long by 2·5 feet wide by 2 feet deep. The tanks had flat perforated bottoms and a 2-inch layer of gravel was put in before filling with sand. Arnon and Grossenbacher (1947) arranged a fall of 1 inch along the 10-foot length to improve drainage of excess solution. Excess solution was allowed to drain to waste and was not re-used. Davidson (1946) has described similar long, relatively shallow beds. The reservoirs associated with the beds were designed to allow a minimum of 2 gallons per square foot of sand surface. Davidson pointed out the relation already noted between particle size and depth of sand. This is an important consideration as shallow beds with fine particle aggregates may be waterlogged or insufficiently aerated, and deep beds with coarse aggregates have insufficient capillary moisture in the upper regions. Shallow beds are useful for growing several plants or species in association in tests on toxicity and accumulation of various nutrients, as studied by Eaton (1942 a) (1944), or for any large-scale tests including work with synthetic resin ion-exchange materials as in that described by Arnon and Grossenbacher (1947).

Tanks and deep sand beds have been used for fruit tree cultures by Lagasse (1929) and these were probably some of the earliest large-scale cultures; they were constructed about 1910. They were adequately drained 12-foot diameter concrete pits which held nearly 23 tons of sand and were probably the largest yet described.

The tanks used by Thomas *et al.* (1943 a) in the individual air-conditioned glass house units were 6 feet square by 2·5 feet deep, glass-lined concrete pits. The pits held 4·3 tons of sand (0·5-0·6 mm. particle size) and were sub-irrigated from glass-lined reservoirs 5 feet by 6 feet by 3·5 feet deep, which held about 600 gallons (2,800 L.) of solution. Water consumption averaged 8 gallons per day per tank (36 square feet of sand).

The tree culture tank beds described by Eaton (1936) were 3 feet by 10 feet by 6 feet deep and were buried in the ground to a depth of 5 feet. The reservoirs were only of 350 gallons (1,600 L.) capacity and 110 gallons (500 L.) were retained in the sand. These beds were later enlarged, Eaton (1941 a) (1942 b), and were similar to, possibly the same as, those used by Hayward and Long (1941 b), Hayward *et al.* (1946). The general arrangement consisted of tanks measuring 5 feet by 10 feet by 6 feet deep, submerged to a depth of 5 feet and spaced to allow 10 feet by 18 feet between centres and shown in Figure 6 G.

The tanks held 12 tons of sand with a water capacity of about 180 gallons (800 L.) and were supported first on a 4-inch layer of gravel and then by the special permeable aluminium silicate bricks overlying drainage channels. These drainage channels were connected by vertical pipes through the gravel and sand to the air above the sand to provide additional aeration. The drainage liquid was led through " duroline," a silica-cement lined piping, to reservoirs set 10 feet below the sand tanks. These reservoirs measured 7 feet by 4 feet by 6 feet deep and held 1,000 gallons (4,500 L.) of solution. The nutrient solution was pumped to the surface of the sand by a 2·5 h.p. pump controlled by time clocks, and distributed evenly from a double line of perforated pipes. The pumps were chrome-nickel alloy with brass impellers. Sand abrasion was avoided by care in placing coarse sand over drainage outlets. Two irrigations were made daily during dormancy and 4 daily during the growing period. A wire netting cage was erected 20 feet above the sand surface. Two peach trees were grown in each tank by Hayward *et al.* (1946) and yielded in a manner comparable with good commercial standards.

The use of a cage makes micronutrient-deficiency studies difficult owing to contamination from drippings from the cage. Millikan (1947 a) reported an instance of zinc toxicity caused by the dripping and deposit from a galvanised cage falling on to flax plants and he refers to the use of bitumen paint at Rothamsted to minimise this trouble. In spite of such precautions contamination is still likely and the substitution of metal by a paraffin-waxed string or rope net erected when required might be worth consideration where attempts to study micronutrient deficiencies are made in the open air. Light screens of glass substitute overhead were used by Mitchell (1939) to afford additional protection.

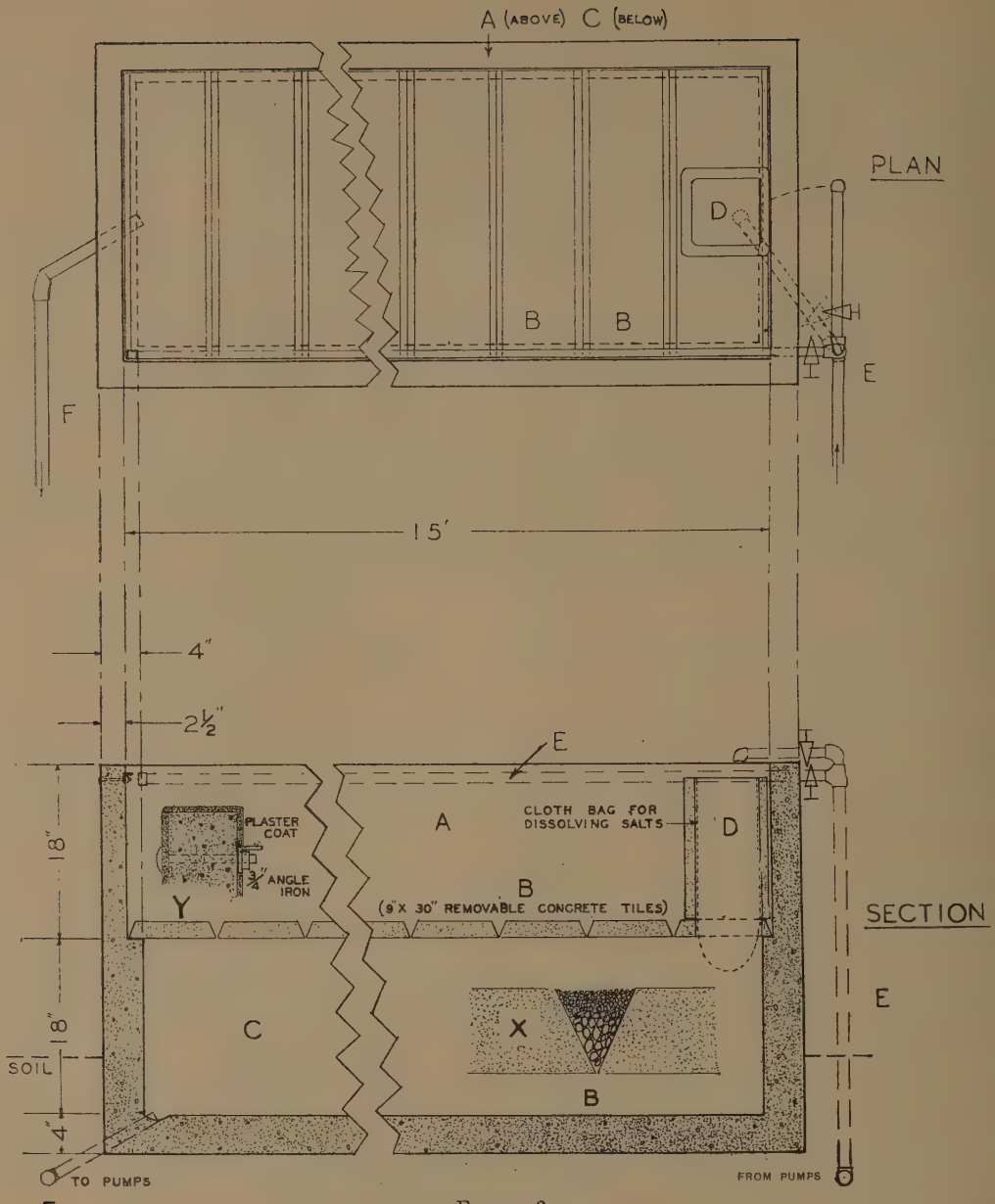


FIGURE 6F

New shallow beds for use with automatically-operated sand cultures (*Eaton*, 1936)

A = Concrete sand bed 15′ × 2′ 6″ × 18″ deep.

B = Bevelled concrete tiles across length of bed, provided with coarse gravel in junctions for drainage and retention of sand (see Inset X). Tiles were later replaced by porous aluminium silicate bricks.

C = Reservoir sections (capacity 1470 L) for each sand bed and precast in same unit.

D = Vertical tile (tube) giving access to reservoir for adding salts or inspection.

E and F = Pipes leading from and to pumps.

X = Inset :—Enlargement showing gravel between tiles.

Y = Inset :—Section of wall and angle iron support covers.

Detailed accounts of large-scale cultures employing large numbers of smaller containers have been given by Vogel (1929) (1931) and by Schropp (1932). Both authors described systems where rectangular enamelled metal containers holding about 11 gallons (49 L.) of sand were arranged in groups of 4 on a base plate held on a trolley. The trolleys were on rails and could be wheeled in a north-south direction. At the ends of the rails ran a cross rail so that a group of pots on the base plate could be wheeled along on to a smaller trolley and transferred to the end of another rail and thence to a different trolley. Each trolley was about 34 feet long and held 24 pots ; each greenhouse unit accommodated 12 trolleys. In Vogel's (1936) arrangement each pot could be weighed *in situ* by mobile scales that wheeled along the trolley and by means of a lever and cam were raised up to take the weight of the pot, or by sliding pairs of pots on to a weighing platform. These outfits now appear somewhat cumbersome in spite of their mechanisation and rather out of keeping with modern layout and methods. The automatically-irrigated sand cultures used by Chapman and Liebig (1938) have been described earlier.

The constructional materials used for tanks, piping and other equipment in large-scale cultures call for comment. Wood may be satisfactory for tanks, but Hoagland and Arnon (1939) pointed out that redwood is often toxic unless treated with bitumen. Bitumen-painted iron was used by Eaton (1931) and was replaced by similar shaped precast concrete tanks in the later work by Eaton (1936). Eaton (1936) also used galvanized tanks for tree cultures and coated the surface with red lead to prevent corrosion. No toxic effects were observed and in further tests with maize no injury was produced by up to 20 p.p.m. of lead acetate. Hooper (1937) found, however, that although 30 p.p.m. lead (as phosphate) produced no visible toxic effects in french beans, dry weights were reduced by concentrations of lead greater than 3 p.p.m. Davidson (1946) regarded asphalt-surfaced or bitumen-painted black iron or concrete as satisfactory non-toxic containers. Eaton (1941 a) and Hayward *et al.* (1946) used a sprayed " static protective coating " of emulsified asphalt to large concrete tanks used to grow trees. Details of asphalts and bitumens and the methods used to apply them have already been discussed under containers, and water storage.

Bronze and brass valves and connections were found to be satisfactory at pH values of 6-8, but zinc toxicity may arise from galvanized piping or zinc alloy pumps as found by Chapman *et al.* (1939) and by Early (1943).

Details of nutrient solutions suitable for these large-scale cultures are given in Tables 19 A & B on nutrient composition. They may be relatively dilute like that used by Hayward *et al.* (1946) and it may be noted here that magnetite at concentrations between 0·1% and 0·5% provides a satisfactory iron supply for many purposes. Tap water has been frequently used to prepare and maintain nutrient solutions, as the quantities needed are great. Demineralised rain water (see Part Two) would be a useful source of water for more refined experiments with certain micronutrient deficiencies. For many purposes rain water suitably collected and stored is satisfactory, without further treatment (see Part Two). Rain water used in the large-scale installations described by Vogel (1929) (1931) was covered by a layer of oil and stored in 8,000-gallon (36 cubic metre) concrete tanks, from which it was raised by a pump to an overhead tank.

SEED STERILISATION, STERILE CULTURES, PEST CONTROL AND THE RAISING OF CULTURE PLANTS

Seed Sterilisation

Seed sterilisation may be necessary either for sterile culture experiments or for growing certain plants liable to seed-borne infection under culture conditions. Seed sterilisation may be somewhat injurious and the use of heavy metal salts such as silver and mercury may introduce abnormalities or unsuspected impurities into the experiment. For these reasons care is necessary in sterilising seeds and where possible the procedure should preferably be omitted, and in any case does not seem to be required for ordinary sand culture methods.

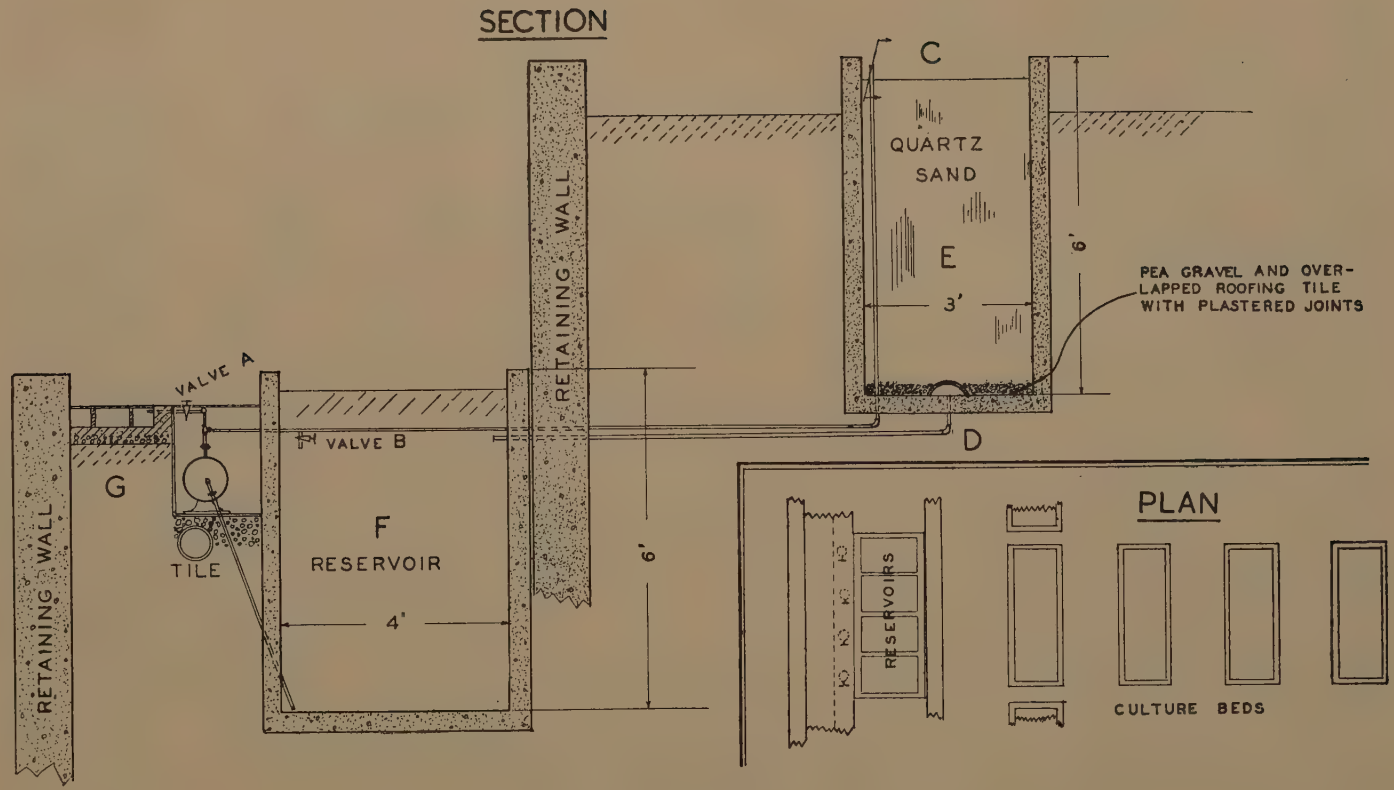


FIGURE 6G

Large scale automatically-operated deep sand cultures (*Eaton*, 1936)

A = Valve opened to discard solution.
B = Valve opened to mix solution.
C = Delivery to sand-bed surface.
D = Return from drainage to reservoir.
E = Sand bed 3′ × 10′ × 6′ deep (asphalt lined).
F = Concrete reservoir (asphalt lined) 4′ × 2′ 4″ × 6′ deep with 1,600 litres capacity.
G = Pump.

Where sterilisation of seeds to be grown in micronutrient-deficiency experiments is required, a volatile agent such as alcohol or hydrogen peroxide (which is readily distilled under reduced pressure in an all-glass apparatus) should be chosen.

Early work on seed sterilisation was reviewed by Wilson (1915). Up to that time absolute alcohol, formaldehyde, phenol, hydrogen peroxide, bromine water, mercuric chloride, silver nitrate and copper sulphate had been variously tried. Wilson recommended calcium hypochlorite containing 2% chlorine as an efficient and relatively non-phytocidal sterilising agent for several seeds. Time of immersion for sterilisation varied from 4 minutes for rape to 15 minutes for wheat. Periods of immersion leading to injury were not more than 2-5 times as long as those for sterilisation. No success was obtained with potato tubers or buckwheat.

Mercuric chloride has been frequently recommended as a sterilising agent, usually at 0·1% by the following : Hopkins *et al.* (1931) (legume seeds, 5 minutes' immersion), Virtanen *et al.* (1933) (legumes ; clover, one minute ; pea, 4 minutes), Hopkins (1934) (*Lemna*, half minute), Barker and Broyer (1942) (squash plants, 1 minute immersion), Gilbert and Shive (1942) (soya bean). Davis (1927) used 0·2% strength for roots of apple trees treated for 2 hours.

Many workers cited above combined the mercuric chloride with a preliminary immersion in 95% alcohol or use of reduced pressure to eliminate air bubbles and to ensure complete surface wetting. Hutchinson and Miller (1909) used 0·25% concentration for 3-4 minutes under reduced pressure for wheat. Tarr and Noble (1922) sterilised maize by immersion for 1 minute in 1% $HgCl_2$ in 50% alcohol. A technique developed for many small seed types by Audus (unpublished) comprises immersion for 3 minutes in 75% alcohol, 3 minutes in 4% mercuric chloride, 20 rinses in sterile distilled water, 10 minutes in sterile water containing trace of $(NH_4)_2S$ and 20 rinses in sterile water. The treatment given by Hopkins *et al.* (1931) was combined with use of Dakin's solution containing 0·5% free chlorine prepared by adding boric acid to sodium hypochlorite. Ghosh and Burris (1950) also used a combination of mercuric chloride, alcohol and hypochlorite, and evacuated during each immersion. Thimann and Edmondson (1949) used aqueous-alcoholic mercuric chloride to sterilise fronds of *Polyrhiza* (*Lemna*).

0·1% silver nitrate solution was used for tobacco seedlings by Beaumont and Larsinos (1929), Beaumont *et al.* (1931) and by Steinberg (1947 a, b, c). The immersion was for 15 minutes. Formaldehyde was used for barley by Richards and Templeman (1936). Immersion for 20 minutes in hydrogen peroxide was recommended by Tausson *et al.* (1944) for seeds of several species. Immersion for 2 hours in saturated solution of " Lemesan " (hydroxy mercuric chlorophenol) followed by rinse in 5% bromine water was used by Stephenson (1942) (1943) for sterilisation of pea, lettuce, and cantaloupe seeds. This treatment was fatal to seeds of Kentucky blue grass and 1 minute in 95% alcohol followed by 1 hour in 2-10% calcium hypochlorite solution was a satisfactory alternative. Seeds sterilised as described were usually washed in sterile water and germinated on sterilised filter paper or cotton wool.

Sterile Cultures for Whole Plants

Early sterile cultures for whole wheat plants were described by Hutchinson and Miller (1909). Water and sand cultures were used and the culture vessels were Wolfe bottles. Davis (1927) set up " sterile " tree cultures, and sterilised sand, pots, water, tree roots and equipment with mercuric chloride, and covered the sand with cotton wool. In this way nitrification of ammonia supplied to apple trees was prevented until mid-June. Street *et al.* (1946) found that nitrification in ammonium type solutions was negligible, if cleanliness and weekly solution change were maintained.

Tobacco seedlings were grown in sterile water cultures by Beaumont and Larsinos (1929) by sowing the sterilised seed on a wick of sterile cotton wool in the neck of a Wolfe bottle. The aerial parts were enclosed in a glass tube plugged by cotton wool. Steinberg (1947 a, b, c) grew tobacco seedlings for 280 days with 2% glucose and a complete mineral supplement, whilst totally enclosed in 250 ml sterilised Erlenmeyer flasks at 25° C. with fluorescent lighting of 500 foot candles intensity. The seeds were sterilised (see above) sown

on sterile filter paper in Petrie dishes and transferred by a platinum loop in a drop of water. The radicle was inserted into the agar.

De Ropp (1946) also described a technique for prolonged sterile culture of totally enclosed whole plants. Provision was made for intermittent irrigation and aeration of the roots by rocking the apparatus so that the sterilised nutrient solution flowed in and out through a sintered disc which supported sand or glass beads as a rooting medium. The aerial parts of the plant were surrounded by a glass chimney of appropriate dimensions with side and top aperture plugged by cotton wool. A separate aperture gave access to the solution reservoir for sampling or replacement. Nilsson (1950) devised a sub-irrigated sterile sand culture holding 250 g. sand. Filtered air was used to elevate the solution and filtered air was drawn through the sand as the solution receded.

An efficient sterile-root water culture technique was described by Barker and Broyer (1942). The 1-L. pyrex Buchner flask culture vessels and similar 3-L. solution reservoirs were filled with 3·5 L. of Hoagland's nutrient solution (Hoagland and Snyder 1933), connected together and sterilised. The ferrous sulphate and tartaric acid were sterilised separately and added afterwards. Seeds were sterilised as described above and sown in sterile agar peptone and seedlings transferred to sterile cotton wool. Aeration was provided by filtered compressed air through sintered pyrex glass aerators. Maize was found difficult to maintain in a sterile condition owing to root infection *via* the leaf sheath after germination. Ghosh and Burris (1950) grew tomato, tobacco and clover in undrained sand cultures holding ·5 kilo sand and 75 ml solution, sterilised for 2 hours at 120° C. Thermolabile materials were sterilised in a Seitz filter, seeds were sown on sterile filter paper. Blanchard and Diller (1950) grew beans with their roots in sterile culture. Seeds were sterilised by Wilson's (1915) method. The culture jars were 9 cm. deep and 9 cm. wide, with screw cap lids. An aluminium cylinder was held in a hole in the lid by the pressure of an aluminium disc against the flange on the tube. The assembly was held in place when screwed down on to the jar. The culture jar and solution were sterilised, and the sterile seed placed in vermiculite in the cylinder, supported on a layer of cotton gauze through which the radicle could grow. Plants were grown to a height of 30 cm. and contaminated cultures were less than 5 per cent.

Other methods, less efficiently aerated and not able to produce good growth, or more involved, have been described by Klein (1938), Virtanen and von Hausen (1931 a), Virtanen *et al.* (1933) and Tausson *et al.* (1944).

Associated Growth Cultures in which two or more different species are grown together have been used to study the influence of one plant upon another and especially the problem of nitrogen donation by legumes. Virtanen (1933), Virtanen and von Hausen (1931 b) used this technique to investigate the ability of non-legumes to obtain nitrogen from leguminous species, grown in sand culture. Trumble and Strong (1937) and Trumble and Shapter (1937) used non-sterile sand cultures for this work and reviewed extensively earlier work on the subject of associative growth in cultures. The provision of nitrogen-free (analysed) sand is an important precaution to be observed in any experiments of this nature. Detailed reviews have also been published by Nicol (1934) (1936).

Pest Control Measures

Records of infestation of culture plants by pathogenic organisms and insect pests are not numerous and few details of their control have been given. Methods used at Long Ashton are given in Part Two.

Wallace (1930) controlled aphis attack on apple trees by tar oil winter wash and use of nicotine applied on a camel's hair brush to foliage during the summer. Woodman (1939 b) controlled aphis attack on lettuce in sand cultures by nicotine fumigation. Hoagland (1941) found that red spider attack on Myrobalan plum grown in refined micronutrient-deficiency cultures could be mainly controlled by spraying with redistilled water. Ramig and Vandecaveye (1950) controlled red spider on raspberries by spraying with water at 45 lb. p.s.i. at short intervals. Hewitt (unpublished) used a purified spray preparation to control aphis

infestation on strawberries in iron and manganese deficient sand cultures. It consisted of 0·1% redistilled nicotine and triethanolamine oleate* at a concentration of 1% as a wetting agent in distilled water from an all-glass atomiser. Pest control in micronutrient-deficiency cultures is difficult owing to contamination of spray materials. Vanselow and Datta (1949) used hand picking or spray materials checked spectroscopically for pest control in molybdenum-deficient citrus plants. Davies (private communication) found 1 p.p.m. Mo in a soap used as a wetting agent. Gauch and Magistad (1943) dealt with thrips on lucerne by fumigation with nicotine, but injury was caused and only incomplete control was obtained. Meyer (1945) controlled thrips on kok-saghyz by painting the leaves with rotenone emulsion. Machlis (1941) found 0·125% nicotine was effective against aphis and thrips on bush bean and Sudan grass.

The most common and serious diseases of culture plants appear to be root rots in maize and citrus in water cultures, powdery mildew in cereals and occasionally in rape, and clovers, *Rhizoctonia solani* in potatoes (blight is not common in culture plants), black aphides in beet, green and grey aphides in *Brassica* species and fruit crops. Aphides are controlled by 0·05-0·1% nicotine sprays, and mildew by colloidal sulphur. Red Spider may attack fruit, including blackcurrents, and also clover.

Methods of Germination of Seeds and Raising of Culture Plants

Considerable attention has been given to devising suitable means of raising large numbers of uniform seedlings for use in culture experiments. Sachs (cited by Hoagland and Arnon (1939) and Birner and Lucanus (1866)) germinated seeds required for water culture experiments in washed or acid-extracted sawdust before transfer to the culture solutions. Sawdust has been used also by Brenchley (private communication) and Rowe (1936) for boron-deficiency studies and appears very convenient. This method has been used in modified form by others for water culture experiments. van Schreven (1936 a, b) germinated beet in leaf mould before transfer for copper-deficiency water cultures, and observed symptoms 19 days later.

Many workers including Brandenburg (1935), Arndt and Hoffman (1937) and Millikan (1942) (1944) who worked on copper and zinc, Löhnis (1937), Brenchley (private communication), and Lehr (1940) who worked on boron, Mulder (1948 a) who studied molybdenum, and Arnon and Hoagland (1940) who reported experiments on crop production, raised seedlings in acid-washed sand before transfer at an early stage of development to micronutrient-deficiency water cultures with evident success. Rapid germination is obtained by this means and contamination is negligible. Large numbers of seeds can be sown and problems of aeration and of infection rarely arise. Moist filter paper in a saturated atmosphere was used to germinate small seeds by Brenchley (private communication), van Schreven (1936 a, b) and Machlis (1941). McMurtrey (1929) raised tobacco seedlings in compost before transfer to culture solutions for boron deficiency.

Knop (1860) germinated seeds in a shallow layer of water until the roots emerged, when they were transferred to netting above spring water. When the shoots emerged the seedlings were transferred to the culture vessel. This method appears to have led to the general procedure for water cultures popular especially in the U.S.A., during earlier years and used in various modifications by many, including Schreiner and Skinner (1910), Tottingham (1914), Shive (1915 a), McCall (1916 a), Salter and McIlvaine (1920), Johnston (1921), Hibbard and Gerschberg (1924), Trelease and Trelease (1926), Machlis (1941), Arnon and Johnson (1942), Piper (1942), Shive and Robbins (1942), Warington (1946). Seeds were soaked for a few hours in water and then sown on, or between, moist filter paper in a humid enclosure or a temperature-controlled germinator. When germinated they were transferred either to a waxed net or gauze suspended above, and just in contact with, extra-dilute nutrient solution, or flowing tap water, or placed on a perforated cork or aluminium raft floating on the surface of the solution, until sufficiently advanced for transfer to the culture solutions. McCall (1916 a, b) used this type of method to raise cereal seedlings before transplanting

* The triethanolamine oleate was kindly prepared by Dr. D. Woodcock by separate distillation of triethanolamine and oleic acid, which were then mixed in equimolecular preparations.

into sand cultures. Hoagland and Broyer (1936) placed seeds soaked for 18 hours on a gauze over water in the dark and transferred the containers gradually to full light after a few days when the leaf emerged. Hibbard and Gershberg (1924) took care even to immerse the vessels of solution supporting the seedlings in thermostat baths and all seed was obtained from a central stock originally entrusted to Dr. B. L. Livingstone. Davis and Hoagland (1928) placed seeds on the netting after a longer preliminary soaking for 16 hours and kept the seedlings in the dark until the roots were in contact with the solution. The seedlings were then grown at low light intensity until 9 cm. high before selection and transfer. van der Veen (1940) germinated coffee seeds on moist filter paper for 4-6 weeks before transfer to water cultures.

Gregory (1943 unpublished) found difficulties in raising flax seedlings by germination on filter paper followed by transfer to a waxed net, as dwarfed tops resulted. Acid-washed sand caused root injury (possibly due to the ion-exchange effect noted in the Section on sand) and successful results were finally obtained by germinating the seeds in contact with vertical strips of filter paper partly suspended in nutrient solution. The response was attributed to improved aeration which may notably affect seed germination. The problems of seed dormancy and " stratification " are complex and have been adequately described by Crocker *et al.* (1948).

Pathogenic infection of seeds germinated in water is not uncommon and it may be desirable to sterilise the seed before use. This precaution is essential for sterile cultures ; the question of seed sterilisation has been discussed.

Methods used for sand culture are often rather simpler than for water culture. In many instances the seeds are most conveniently sown directly into the sand cultures and thinned afterwards by removing unwanted seedlings at an early stage or as soon as evidence of variability or the presence of rogues can be detected, cf. for example Meyer (1945) (1946). This method provides a more uniform stand than transplanting into the cultures in the opinion of Gregory and Crowther (1928) and also of the writer. For some plants, however, where only one specimen is desired in each pot, it may be better to raise seedlings in sand or compost and transplant. This method is suitable for many *Brassica* species and tomato, as stated in Part Two and by Shive and Robbins (1942) and Robbins (1946). Legumes, cereals, beet and most root crops are best sown direct (cf. Part Two). For exacting techniques with micronutrient deficiencies the seeds can be sown in highly purified sand supplied with the appropriate deficiency nutrient until large enough to transfer. This avoids any need to wash roots or break up the ball of sand and the transplanting check is only very slight. Spencer and Shive (1933) found that rhododendron seedlings raised in sand were less liable to disease and grew more vigorously than when raised in compost.

When seedlings are transplanted they should be set slightly deeper than their level in the seed box. The sand should be flooded and jolted lightly to settle sand firmly around the roots. This procedure is especially recommended by Robbins (1946) and in Part Two. The importance of using only " new " seed cannot be over-emphasised.

Certain plants, especially trees, e.g. citrus and apple, cannot be raised by seed if uniform material is required, especially if particular rootstocks are required. The usual method for citrus is to raise cuttings in sand and transplant when well rooted to either water or sand cultures. This procedure involves a larger reserve when micronutrients are considered, but was used by Chapman *et al.* (1937) for zinc deficiency and by Liebig *et al.* (1942) (1943 b) for studies on effects of aluminium and gallium, and by Vanselow and Datta (1949) for successful molybdenum deficiency tests.

Wade (1944), however, raised Valencia orange seedlings from seed in purified sand for work on copper deficiency, and Chapman and Brown (1943) used sweet orange and grapefruit seedlings, but raised Eureka lemons as cuttings. Smith and Bayliss (1942) raised *Pinus radiata* seedlings from seed of zinc-deficient trees for zinc deficiency cultures. de Haan and Schoorel (1940), de Haan (1941) raised tea plants from seed germinated in sand and later transplanted.

Pineapple is often raised as a " slip " by rooting a leaf cutting for a month either in tap water as described by Sideris *et al.* (1943) or in sand as described by Schappelle (1942) before

transfer to water cultures. Hart (1934) raised sugar cane by cutting healthy cane to " 3-eye " pieces, which were immersed in water at 52° C. for 20 minutes to kill stalk mite before setting in sand with tap water. The rooted cuttings were later divided with about 1 inch length of " eye " still attached. Single leaf cuttings from clonal stocks of cacao were raised in sand cultures by Evans (1950) at Trinidad (also private communication from Dr. E. J. Maskell). The cuttings were dipped in a solution containing indole butyric or indole butyric and α naphthalene acetic acids (1 : 1), 5-7 mg. per ml. of 50% alcohol and placed in a propagating frame. The optimum temperature was 27-30° C. and shading 20-25% of full sunlight. The most critical factor, however, was the air/water relationship in the rooting medium. Fine sand required watering less frequently than coarse and unfavourable conditions due to excess moisture (poor aeration and waterlogging) caused rotting, or abnormal callous formation. Excessive aeration caused a callous plate to form over the cutting which delayed rooting. The nutrient and carbohydrate status of the parent plant were also important factors. 50-60% sunlight in the nurseries was optimal and 80-90% single leaf cuttings taken as described rooted in 17-23 days. Evans (1950) also described a technique for storing cocoa beans in a viable state and the method for raising seedlings. Storage of beans is achieved by layering them in charcoal containing 35% moisture in split tin boxes with access to air. Seedlings are separated from the charcoal by separating the halves of the boxes. They are placed in freely drained sand saturated once with water and covered over. No further water is given until leaves have unfolded when the covers are raised by stages. Waltman (1931) raised strawberry plants in sand culture by rooting " runners " from a parent plant. It was found necessary to allow the runner to become well rooted before severing the stolon.

Avocado trees were raised by Furr *et al.* (1946) as 1-year-old grafts on seedling rootstocks. Roots were washed before transplanting into 8-gallon sand cultures. Deficiencies of most mineral elements were produced in 1 or 2 years from planting.

Apple trees have been raised in nursery beds on appropriate stocks and fibrous roots were generally severely pruned and washed before transplanting by Wallace (1922) (1924) (1930) and Nightingale and Blake (1934 a). Trees raised thus naturally have a considerable reserve of nutrients in spite of root pruning. This objection might be overcome by layering rootstock plants in purified sand in the absence of selected micronutrients followed by budding or grafting the desired scion when the stock has rooted. Robbins (1946) stated that solutions used during rooting of cuttings should be low in nitrogen.

Potato plants are best raised from whole tubers weighing between 30 g. and 80 g. and planted about 4 inches deep in sand cultures. The effects of separating sprout from seed piece is discussed later and is not generally recommended. Johnston (1920) sprouted and rooted tubers in moist sawdust for as long as 7 weeks before separating the shoot and roots from the tubers and transplanting into sand cultures. The maximum yield of dry weight of a single plant including tubers was only 94·6 g. compared with yields frequently greater than 500 g. at Long Ashton. Street *et al.* (1946), in a study of nitrogen assimilation, used detached potato sprouts. These were obtained from tubers sprouted and rooted in moist sand. The tubers were trimmed by removal of a thin slice from the heel-end, according to the method of McDermott (*loc. cit.*) to hasten sprouting, set on to moist sand, and buried for 20 days. The rooted sprouts were then excised for the experiments. At Long Ashton tubers are sprouted after first washing them. The tubers are set on end in trays in the greenhouse in the light with the " rose " end uppermost. Dark green stocky sprouts reach a suitable stage of development in about 4-5 weeks. Separation of the sprout after rooting may be necessary for some purposes, but is not required for manganese, iron or boron deficiencies. Unpublished work by Hewitt and Jones showed that the yield from potato plants progressively diminished as the proportion of tubers attached to sprouted shoots was reduced. In other work by the writer, however, good yields were obtained from plants raised after removing the tubers from well-rooted and elongating shoots. Bolas and Portsmouth (1948), Bolas and Ruck (1949, unpublished) showed that potato leaves could be rooted in 2·5% naphthalene acetic acid solutions (24 hours). Control leaves also rooted and all leaf cuttings were liable to excessive insect attack.

Newton (1923) devised a special " germinator " to obtain rooted potato shoots for use in water cultures. A large cylindrical vessel was lined with wet felt and a central " island " was lined in the same way. Tubers were set on the " island " and maintained in a dark, saturated atmosphere for 4 weeks, when good root systems were produced. Variability was reduced by using large tubers able to supply several " cuttings." Yields in water culture were poor, however, and only attained 30 g. including tubers. It is evident that potatoes are not well adapted for growth in water cultures and support of the tubers is a problem only partly solved by the nylon basket used by Houghland (1947). Houghland rooted small tuber cuttings in sand before transfer to water cultures. Appleman (1918) found that sprouted tubers gave greater yields than dormant ones and were further improved if also rooted before planting.

In experiments where *Lemna* and related species are used the plants are isolated as a single frond and continuously subcultured as such in a standard medium, cf. Ashby (1929 a, b), Ashby *et al.* (1928). The frond is sterilised when necessary, by immersion for a few seconds in alcohol followed by mercuric chloride 0·1% according to Hopkins (1934), Saeger (1937) and Steinberg (1941) (1943) (1945 b).

Marchantia may be propagated and maintained from subcultured gemmae or by other means used by Voth and Hamner (1940) in culture experiments on this plant. *Aspergillus niger* is raised and subcultured from one or a few drops of a spore suspension, prepared with sterile water. The original culture should be derived from a single spore, as specified by Steinberg (1945 a) and Mulder (1939 b) (1948 b). The methods of Flemion (1938) (1941) should be consulted when it is desired to determine in advance the viability of seed.

COMPARATIVE ASPECTS OF SAND AND WATER CULTURES AND CHOICE OF TECHNIQUE

Comparative Aspects of Sand and Water Cultures

The kind of equipment needed for sand and water cultures differs, and the choice between the two methods must be made at the outset. The relative merits and problems of the two methods are therefore briefly compared.

Sand cultures provide a solid rooting medium and in this respect approximate more closely than water cultures to soil conditions ; the plants are also supported naturally by their root systems, which grow in the dark. For these reasons it is generally easier to manage plants grown in sand culture, especially if they have to be moved. Where trees, heavy plants or tubers are concerned the advantages of natural anchorage are still more obvious. It is often not necessary to darken the containers used in sand cultures, but on the other hand cursory examination of the roots is not possible during the experiment. The fact that sand culture plants are anchored in the aggregate, however, makes the provision of covers to exclude dust more difficult, especially for cultures containing plants with several shoots or more than one plant. This point has been dealt with under " containers."

The water supply in intermittently watered sand cultures depends on water retention in the pores, and they thus require more frequent attention than water cultures. This point is far outweighed by the superior aeration freely obtained in sand cultures due to the aspiratory effect of the gravitating fluid. The evidence of comparative experiments is greatly in favour of the yields and growth of plants in sand. A great part of this superiority is probably due to the improved aeration produced, as suggested by the work of Hall *et al.* (1914 a, b), Barker (1921), Allison (1921), Allison and Shive (1923), Hoagland and Martin (1923), Arnon and Hoagland (1940), and sand cultures require no additional aeration so frequently needed in water cultures. This eliminates a great deal of equipment needed to provide forced aeration and also avoids injury from over-aeration. Where sealed (poorly aerated) sand cultures have been used the superiority over water cultures may be lost, as in the experiments of Bakke and Erdman (1923), although this was not the experience of McCall (1916 a).

Sand cultures are usually free from the incidental chlorosis due to non-availability of iron compounds which is a frequent trouble in water cultures. This is partly due to the

retention of precipitated iron compounds on the sand particles, where they remain available instead of falling to the bottom of the culture vessel out of reach of the roots. Another reason has been suggested by Went (1943 a) who considered that the development of roots in a saturated atmosphere or a moist solid substrate was conducive to the production of " rhizocaline " and freedom from chlorosis. In even relatively high grade sands it is often difficult to produce iron deficiency until rigorous purification has been carried out. Many sparingly soluble compounds, such as calcium carbonate, iron and aluminium phosphates and magnetite, can be used as sources of different elements for sand cultures, as detailed elsewhere.

Root contact and ion-exchange phenomena can be studied more easily in sand cultures, which are especially adaptable to supply of nutrients in the form of absorbed ions on exchange media discussed in the Section on solid rooting media.

The close control of nutrient composition and pH are perhaps less readily accomplished in sand cultures than in water culture. Thus Nightingale (1934) found that pH gradients from root to medium were much greater in sand cultures than in water cultures. This can be attributed to the relatively restricted diffusion in sand cultures, where the actual quantity of nutrient solution in the pore spaces surrounding the roots is relatively small compared with water cultures. These gradients persist even when continuous renewal of solution is employed until high rates of flow of 20-30 L/day are used. Breazeale (1905) concluded that more concentrated solutions were required for optimum growth (determined by transpiration) in sand than in water culture. Chapman and Liebig (1940) similarly found that low concentrations of nitrate were adequate for citrus in aerated (stirred) water cultures, but not in sand cultures. Presumably restricted diffusion renders leaching necessary in sand cultures owing to accentuated unequal absorption. Solution change is less important in water cultures provided that water loss is restored and adequate nutrient content maintained. Hoagland and Martin (1923) pointed out that comparisons between sand and water cultures should be made on the basis of equal volumes of solution, i.e. a sand culture holding 10 kilos and is comparable with a water culture of 1-2 L. according to moisture content of the sand. Under these conditions of comparison barley was found to produce nearly double the amount of dry weight in sand as in water culture provided the solutions were renewed frequently.

An important advantage of water culture is the avoidance of contamination due to the presence of micronutrients in the sand. The efficient removal of these from sand presents difficulties and special equipment is needed for work to be carried out on any but a small scale. It is also difficult to be certain that every trace of the impurity has been eliminated from the sand and the search for yet unrecognised micronutrients is correspondingly more difficult.

Water cultures permit more conveniently the change of nutrient treatment at specified times, since it is only necessary to replace the old solution or to transfer the plants to other containers, and the roots can be thoroughly rinsed if desired. Sand culture plants, however, can only be leached prior to change of treatment or else uprooted and transplanted with almost inevitable check to growth except in the earliest stages of growth. Leaching may be satisfactory to effect changes in treatment with respect to macronutrients, but is less satisfactory for micronutrients, although such methods were used by Eltinge (1936), Walker (1941), Chandler (1941) and Reeve and Shive (1944) with complete success for boron deficiency.

Greater latitude in concentrations of toxic or potentially toxic elements is permissible in sand than in water cultures. The threshold concentrations for toxicity from metal ions such as copper, zinc, lead, nickel, in sand cultures may exceed by a factor of 10 or thereabouts those permissible in water cultures, cf. Jensen (1907), Jacobsen and Swanback (1929), Scharrer and Schropp (1933) (1936), Bortner and Karraker (1940). A similar difference applies to injury at low pH values ; Arndt (1922), McCall (1923). Accidental injury from abnormal nutrient composition and toxic metals in water supplies is also thus less likely. Presumably the difference relates to restricted diffusion discussed above and possibly in some instances also to adsorption on the sand. Although the concentration of elements causing toxicity may vary according to the type of culture the optimum composition of nutrient solutions does not appear to change appreciably according to Shive (1918 a), Shive and Martin (1918 b),

and as might be inferred from the generally similar nutrient composition used for both methods (Table 19). Prianishnikov and Domontovitch (1926) found, however, that whereas yields of barley in sand and water cultures were similar with Crone's and Hellriegel's solutions, their own formula, which included $CaHPO_4$ and NH_4NO_3, produced markedly inferior growth in water culture.

It is evident that special advantages attach to both sand and water culture methods for studying particular problems. In the opinion of the writer sand cultures are probably simpler to manage and appear to provide a more satisfactory growth medium for most plants, for reasons indicated. Where the choice is not determined by special needs, sand cultures are preferable and experience has shown that micronutrient deficiencies of iron, manganese, copper, zinc, boron and molybdenum can all be produced in acute form in this medium provided that suitable measures are taken to purify the sand. The evaluation of newly-identified or potential micronutrients and the sudden elimination of micronutrient supply during growth may be more readily accomplished in water culture.

The Choice of Plants for Culture Experiments

The selection of a suitable crop plant for experimental work is often limited if the problem concerns a particular species or type as, for example, a cereal or root crop. When this restriction does not apply other factors may be considered. Thus it is often useful to use a crop about which much is already known, for example soya bean, tomato, barley or maize, so that abnormal effects may be readily recognised and advantage taken of previous experience and data.

Borthwick (1946) has pointed out that culture plants should preferably be photoperiodically " indeterminate," e.g. the tomato and certain varieties of soya bean (quasi-indeterminate). Shive and Martin (1918 a) chose buckwheat for work on nutrient requirements as this plant has two growth phases of almost equal duration and is fast growing. Grossenbacher and Livingstone (1936) used sorghum for repeated experiments because of its capacity for good growth under glass at all seasons. Robbins (1946) suggested that wheat, oat, lettuce and radish are suitable for work during winter months, as they are relatively less sensitive to reduced light intensity than many crops. Strains of tomato plants able to fruit in winter may also be useful at such times.

In some experiments a pure line of a particular species has been used almost exclusively, for example Plumage Archer barley by Gregory and his associates. This method assists in maintaining reproducibility and allows more reliable comparison between results obtained at different times. Sugar beet, on the other hand, is unfortunately a highly variable subject, at least where leaf morphology is concerned. Robbins (1946) pointed out that clonal stocks are even less variable than pure seed lines and recorded that clover, tomato and lucerne were suitable for raising as clonal stocks by vegetative propagation. Physiological and morphological uniformity may allow of lower replication, especially advantageous for greenhouse work. Evans (private communication) preferred clonal leaf cuttings of cacao.

Varieties resistant to certain diseases may also be selected as, for example, the use of " Sacramento " barley by Hoagland and Broyer (1936) on account of its freedom from mildew, which is usually a disadvantage associated with the use of many cereals. Brassica crops, similarly, are usually attacked by aphides and caterpillar. Tomato, except for white fly, and potato remain reasonably unblemished in culture.

In many experiments comparative responses are often of interest and the choice of crops may then extend to cover a range of probable characteristics. For example rice and some other cereals have been used to represent contrasting types with respect to ammonium and nitrate nitrogen or iron nutrition ; similarly, wheat and barley or oat and rye are useful pairs for studies of soil acidity problems owing to their widely different susceptibility in the field. Swede, dwarf bean and sugar beet may be chosen to represent crops that are respectively relatively sensitive to excess of manganese, but not aluminium, sensitive to both metals, and sensitive to excess of aluminium but not manganese.

Susceptibility to macro- and micronutrient deficiencies varies greatly between species and even varieties and so also does the response to excesses of the latter. Cereals are relatively susceptible to phosphorus, potassium, copper, zinc, iron and manganese deficiencies, but not to calcium, boron or molybdenum deficiencies. Resistance to boron deficiency is characteristic of many monocotyledons. Brassicas and tomato are well suited to the study of all mineral deficiencies. Brassica crops, lettuce and tomato are especially susceptible to molybdenum deficiency. Schropp (1941) lists some tropical plants useful as indicators of boron deficiency. Mullison and Mullison (1949) tested a wide range of varieties of tomato, cucumber, chard, cauliflower, rape, mustard, lettuce and other vegetables for suitability as vegetables under semi-tropical conditions on Curacao (12° N.). High night temperatures were thought to be the limiting factor to satisfactory growth. With the exception of lettuce which failed to " head," varieties of each crop were found that were suited to the environment which included mean relative humidity 74%, mean minimum temperature 70-77° F. and maximum temperature 96° F. Insect pests were the main problem under these conditions.

The use of *Lemna* and similar plants merits comment. *Lemna* presents advantages for certain types of investigation. It is a representative of chlorophyll-containing higher plants that can be grown in small cultures under closely controlled conditions or sterile culture, and is ecologically suited to water culture. Techniques have been described in detail by Ashby *et al.* (1928) and Ashby (1929 a). As far as is known *Lemna* behaves similarly to other higher plants as regards its mineral requirements. The great number of fronds that can be obtained in a short period from a single plant inoculum facilitates the measurement of growth rate and also retains the advantages of clonal propagation. The small size of the plant reduces the consumption of nutrient solution, and extends its usefulness as a sensitive test organism for the presence of micronutrient elements. This aspect of the use of *Lemna* has been discussed by Steinberg (1941) (1943) (1945 b). On the other hand, problems of translocation, immobilisation at nodes, and morphological differentiation are all simplified as compared with most higher plants and direct surface absorption may introduce a new factor. The significance of these aspects of plant behaviour in mineral nutrition require consideration when data obtained with *Lemna* are discussed, and conclusions deduced from such results should not be applied to more highly organised species without due caution and reservations.

Microorganisms, especially *Aspergillus niger*, have been referred to frequently in these pages, but solely with the purpose of illustrating points of technique. The use of such organisms has been advocated on grounds similar to those advanced in support of *Lemna*, namely small scale, highly refined technique and, if required, closely controlled environment. It must always be remembered that these organisms differ from higher plants in several important respects that may invalidate attempts to apply the results obtained with them to the nutritional requirements of higher plants. Lack of chlorophyll and reliance on heterotrophic carbon assimilation, lack of specialised absorbing organs and simplified morphological differentiation require consideration. Their apparent independence of boron supplies also indicate an important difference from higher plants. In some respects, however, e.g. nitrogen metabolism and molybdenum requirements, results obtained with fungi may help in studies of higher plants.

The Choice and Limitations of Technique

It is evident that the degree of refinement and elaboration of technique used is determined by the nature of the work to be undertaken. It is not justifiable, in the opinion of the writer, to indulge in over-elaborate and highly refined methods for the study of problems clearly relating to macronutrients, provided the following conditions are fulfilled :—

(1) The materials must be sufficiently pure or unreactive to ensure freedom from toxic effects.

(2) They must not provide appreciable amounts of nutrient elements in an indeterminate manner. Each material should be tested to find if contamination is likely to arise from its use.

(3) The nutrient medium must supply *all* the known nutrient elements, except where an omission is purposely made.

The conditions given here are generally fulfilled quite simply by the use of bitumen-treated containers, clean (water- or dilute acid-leached) silica sand, rain water suitably collected and stored (and if possible, demineralised also), or in some circumstances tap water, and recrystallised grade nutrient reagents. When these conditions concerning toxicity and nutrient composition have been satisfied, the choice and treatment of culture materials may require further refinement for special purposes as for example studies of micronutrient problems. For experimental purposes the micronutrients can be conveniently grouped into the relatively " easy " elements—iron, manganese and boron, and the more " difficult " ones—copper, zinc and molybdenum. Water from tinned copper stills or demineralised rain water, bitumen-painted or certain plastic containers, analytical reagent grade salts (either recrystallised or purified by phosphate absorption) and acid-treated sand normally give satisfactory results for the first group. For the more " difficult " elements, water redistilled from pyrex or hysil glass, and similar glass containers are usually necessary for success. Sand may need more prolonged acid treatment. Nutrient reagents usually require specialised purification for experiments on molybdenum deficiency ; treatment by phosphate absorption with a dithizone extraction is satisfactory for copper and zinc deficiencies.

The details relating to a few proved and well-known techniques have been described in the relevant sections. They include the water culture techniques for boron deficiency used at Rothamsted by Brenchley (private communication), for copper, zinc and manganese deficiencies introduced by Stout and Arnon (1939), and for copper and manganese deficiencies devised by Piper (1941) (1942), and methods suited to the study of deficiencies of any of the known micronutrients in sand culture given in Part Two. It should be possible with care to superimpose on most of these techniques the elaboration or modifications required for automatic operation, aeration, continuous nutrient flow or other aspects of culture technique, so long as the essential problems of contamination due to the nature or quantity of materials, and to extraneous sources are carefully studied and overcome.

The ultimate limits of these techniques cannot be easily determined. Experience at Long Ashton has allowed some conclusions to be drawn with regard to the routine limits of contamination in molybdenum deficiency in the refined materials by use of *A. niger* as a test organism. These are probably of the following order :—

Residual available molybdenum
- in sand : 0·01-0·02 micrograms per kilo.
- in water : less than 0·003 micrograms per litre.
- in reagents : less than 0·002 micrograms per litre.
- in containers : not readily ascertainable as these are used in all experiments including the bio-assay.
- in dust : uncertain but may be comparable with amount in sand.

Total for a culture holding 8 K. sand : 0·1-0·2 microgram plus 0·002 microgram per L. of nutrient solution (about 0·1 microgram per season).

Chemical analysis and yield data indicate that individual plants may only absorb between about one-tenth and one-half of this total before being killed by the deficiency.

Arnon and Stout (1939 a) have pointed out that it is not accurate to refer to any element as being non-essential. It is only possible to state that, if certain precautions are taken and negative results are still obtained, then the element in question can only be required in amounts less than those introduced by the technique used. Unfortunately, data similar to that given above for molybdenum are hard to obtain and usually can only be deduced from results of biological assay which often far exceed known chemical methods in sensitivity. If the assay results are themselves negative (as for example is generally found with microelements not yet identified as essential), it is not possible to specify the actual limits of impurity present The problem will remain indeterminate until a positive result can be obtained, and it can only be said that the limits are below chemical detection and may be above biological needs.

THE INFLUENCE OF ENVIRONMENT AND BIOLOGICAL FACTORS ON THE BEHAVIOUR OF CULTURE PLANTS

This section illustrates some of the ways in which environmental factors may affect the requirements of culture plants and the methods that have been devised to secure controlled environment or to account for the variations due to environmental factors.

The Influence of Environment

Light

Light intensity and duration are important factors to be considered in all culture work. The light intensity (and quality) is reduced under glass to varying degrees according to the alignment of the house and the cleanliness of the glaze. Stone (1913) found that reduction of light was 13-18% through clean glass and 28-36% through a badly glazed, old or dirty glass roof. Aluminium interior painting materially improved lighting conditions. Best conditions were secured by large panes and an angle of not less than 60° with the incident light. Loss of light was 14-15% in June and July and 48% in December. More total shade was cast in a house running North-South than in one running East-West. There was no difference in light intensity between 5 and 50 feet from the glass. Light intensity in different parts of a large house may vary greatly. A recent study of these problems in relation to horticultural practice has been published by Lawrence (1948).

The effect of different light intensity on response of culture plants is considerable, as was recognised by Pfeffer (1918). Shirley (1929) found that any reduction in light under glass reduced dry weight yields, whereas reduction up to 50% of midsummer light was without effect in the open ; reduction of light in the open during late summer, however, depressed yields and there was considerable variation between different species. Light intensity was found by Blackman and Rutter (1949) to affect the distribution and accumulation of nutrients by bluebell (*Scilla non-scripta*) in a complex manner.

High light intensity increases the requirements of many culture plants for iron or accentuates chlorosis as found by Gericke (1925 a), Davis and Hoagland (1928), Gilbert and Shive (1942), Shive and Robbins (1942), Gregory and Brenchley (1945 unpublished data). Colwell (1943) and Eaton (1944) found that boron deficiency symptoms were also accentuated by extra light, and boron toxicity is correspondingly reduced in high light intensities. Long days also similarly accentuate boron deficiency, which may not be marked under short day conditions, according to Warington (1933), Skok (1941), Colwell (1943), Eaton (1944) and MacVicar and Struckmeyer (1946). Zinc deficiency may be more acute in bright light Chapman *et al.* (1937) or masked during winter Hoagland (1944), although Trumble and Ferres (1946) observed contrary behaviour. Manganese accumulation is stimulated by high light conditions according to McCool (1934), and Gregory (1943 unpublished) found that manganese deficiency may be more marked during dull weather. Johnston and Hoagland (1929) and Turner and Henry (1939) concluded that high light intensities may reduce potassium requirements. Arnon (1937) and Robbins (1946) found that high light intensity reduced ammonia toxicity. Wallace (1943) and (unpublished) and Hewitt (unpublished) found that nitrogen-deficiency effects may be accentuated by higher light intensity and lower temperature. The relation of light to nitrate assimilation has been reviewed by Burström (1945).

Borthwick (1946) emphasised that great care is necessary to ensure valid results in any study of photoperiod and this applies equally to studies on the relation of nutrition to photoperiod response. Borthwick pointed out that a nightly visit to a greenhouse where a light might be used for as short a period as 1 minute or so may be sufficient to upset the photoperiod response of soya bean or *Chrysanthemum*. When photoperiod sensitive plants are used, the photoperiod may be lengthened or shortened by simple means to produce the required type of development, e.g. flowering. When the photoperiod is shortened, care should be taken to reduce light intensity below 0·01 foot candle. A double layer of black cloth and an extra layer

of white (to reduce heating) supported on frames suffice. The extra dark period should be divided between morning and evening. Supplementary light of 100 foot candles' intensity is usually adequate to extend the photoperiod. Borthwick suggested that absorption of nutrient might be related to photoperiod. Clements (1928) concluded there was an interaction between length of day and solution type, but micronutrients were not given.

Temperature

Root and air temperatures may affect the growth responses of culture plants. Brenchley and Karak Singh (1922) found that wide temperature ranges were tolerated if the minimum temperatures were low enough. Low root temperatures partly compensated for high leaf temperatures. A combination of high light intensity and high root temperature was the most harmful. This was confirmed by Gregory and Brenchley (1945 unpublished data). High light intensity was tolerated if root temperatures were lowered to about 60°-65° F.. Overcrowding was deleterious in spite of adequate water and nutrients, but overcrowded plants grew better than well-spaced ones under unfavourable light and temperature conditions.

It is significant that although maximum growth of tomato was obtained by Went (1944) at 26·5% C. under constant temperature conditions, still more growth was obtained by maintaining day temperatures at 26·5° C. and allowing night temperatures to fall to 17-20° C. The diurnal temperature range in a greenhouse during the growing season may therefore be distinctly beneficial. Robbins (1946) considered that the maximum greenhouse temperature during the day should not exceed 32° C. (90° F.). If necessary some loss of light due to shading was regarded as justified to reduce summer temperatures. Higher day and night temperatures could be tolerated during the summer owing to the greater light intensity. The recommended difference between day and night temperatures was about 6-11° C. During winter, day and night temperatures of 19-28° C. and 13-16° C. respectively were satisfactory for tomatoes. Robbins pointed out that night temperatures during the winter should not exceed the maximum suggested owing to the effects on carbohydrate depletion. Bolas (1933) found that the optima of light intensity and temperature required for maximum assimilation rate by tomatoes were interrelated and were positively correlated. At 1,000 foot candles (approximately normal sunlight under glass in summer in England) the optimum temperature was about 90° F. Observations of Haas (1932 b) and Hewitt (unpublished) suggest that many plants, including citrus and vegetables, tolerate temperatures considerably higher than those mentioned by Robbins (1946) and satisfactory growth has been obtained at 100° F. and even at 105-110° F. Gericke (1921) concluded that potassium requirements were greater at 17° C. than at 28° C., whereas phosphorus requirements were affected in the opposite manner. These results were obtained in solutions devoid of additional iron and might be explained in terms of iron requirements as iron deficiency may be more prevalent at low temperatures, according to Chapman *et al.* (1947). High temperatures and rapid transpiration accentuated salt toxicity in experiments made by Ahi and Powers (1938) and Magistad *et al.* (1943). Gericke and Tavernetti (1936) recommended use of heating culture solution to 80° F., but this was not confirmed by Arnon and Hoagland (1940) and is contrary to the findings of others cited above. Colwell (1943) concluded that boron deficiency was not greatly affected by temperature.

Humidity

Comparatively little is known about the effects of humidity on responses to nutritional factors. Gericke (1930) stated that high humidity is necessary for grain formation in rice. Nightingale and Mitchell (1934) found that humidity modified the response of tomato plants to nitrogen deficiency which was accentuated at low humidities. Robbins (1946) recommended a relative humidity between 50% and 90%, but not to exceed 90%. Fungal leaf infections may be expected to be more widespread under conditions of high humidity. Xeromorphic or other morphological characters may also vary with different humidities. High humidity causes leaf guttation which may contaminate neighbouring deficiency cultures.

Seasonal Effects

The seasonal trend is important as experimental results can only be compared for the same time of year. Yields may vary widely as shown by data of Davis and Hoagland (1928) for lettuce :—

Period	Nov./Dec.	Dec./Jan.	Feb./Mar.	Mar./April	May	Aug./Sept.
Yield dry wt. g.	25·7	15·2	39·9	46·2	97·5	86·9

Brenchley (1916) found that dry weight yields of barley in January-February were only 25% of those obtained in April-May. Johnston (1921) found a fivefold increase for buckwheat from January to June. Outdoor cultures may show iron deficiency in winter due to low temperatures, already mentioned, cf. Chapman *et al.* (1947).

The whole subject of environmental influence on growth is extensively dealt with by Crocker *et al.* (1948).

Controlled Environment Equipment

Many types of controlled environment chambers and greenhouse units have been described and full control of temperature, light, humidity and atmosphere as well as of nutrient supply have been provided by Davis and Hoagland (1928), Went (1943 b) and Crocker *et al.* (1948).

It is obviously impossible to describe the full details of these arrangements, which can only be appreciated by study of the original papers. Early methods for providing some control of light, temperature and humidity were described by Gregory (1921) (1928 a) and Tottingham (1926). Temperature control only received most early attention from Jones (1922), Leukel (1924), and Peltier and Goss (1924), who concentrated on the production of the early constant temperature soil tanks. A simple, inexpensive type of soil tank has been described more recently by Campbell and Presley (1945), for operation between 40 and 110° F. A modification of the Jones Wisconsin Tank, adapted especially for sand culture work in relation to root temperature, was described by Link (1935). Tint (1945) designed a series of relatively inexpensive chambers to operate between certain diurnal temperature ranges varying from 9-11° C. to 29-33° C. and arranged so that there was a gradient from one end of the set to the other with progressive cooling of the air as it passed from the warm to cold end.

Relatively simple means of humidity control have been described by Stoughton (1928) and Wallace and Bushell (1945), and an automatic device for opening and closing greenhouse windows has been used by Heath and Whitcher (1945). This was operated by a weight comprised of slowly self-emptying buckets filled with water by a pump thermostatically controlled. Temperatures were maintained at 27-30° C. during hot weather. Other temperature-controlled chambers have been described by Wilson (1937) and Brown (1939) and by Nightingale (1933), Nightingale and Blake (1934 a, b), Nightingale and Mitchell (1934) in experiments on apple trees and tomato.

Efficient and closely-controlled environment equipment requires special note. Ashby *et al.* (1928), Ashby (1929 a) described the equipment needed for full control of environment, including atmosphere, solution and aeration, for *Lemna* which represents the smallest scale for precise work with higher plants. Fogg (1948) described a simple, constant temperature illuminated bath for work with algae. Davis and Hoagland (1928) designed a fairly inexpensive but accurately-controlled environment chamber for small-scale work with plants such as cereals in water cultures. Growth was reproducible to within 3%. Provision was made for lighting with high intensity of 2,600 foot candles and the water screen was replaced by air cooling owing to troubles with dirt and condensation. Iron requirements appeared to be high. Independent top and root temperatures, humidity control and air, or other atmospheric circulation at 400 cu. ft./min. were provided. Stoughton (1930) used a rather less elaborate set up and much lower light intensity of only 800-900 foot candles. Steinberg (1931) built equipment similar to that of Davis and Hoagland. Light intensity here was still greater at 4,200 foot candles. Steinberg gave special attention to the shape of the spherical water screen which allowed remarkably even illumination. Air flow at 220 cu. ft./min. was provided. Temperature and humidity controls were elaborate and accurate to 0·5%.

Mitchell (1950) described briefly a cabinet in which plants could be grown with a light intensity of 2,000 foot candles at 18 inches from the lamps over an area of 5 square feet. The light was provided by 12 150-watt tungsten filament lamps and 12 3-foot blue fluorescent tubes. The spectrum was continuous and approximated to sunlight. Chance's ON 20 heat-absorbing glass was placed below the tungsten lamps. Temperature control between 40° and 75° F. ($\pm 2°$) was obtained by thermostatic heating and cooling of an air current. The light intensity was thought not to attain the maximum to which *Lolium* will respond.

Large-scale Installations

Large-scale equipment is relatively costly, as difficulties of control of all factors are increased. Thomas *et al.* (1943 a) designed individual air-conditioned, gas-tight greenhouse units to house large-scale sub-irrigated sand cultures. Humidity, temperature and atmospheric composition were all fully controlled.

Went (1943 b) installed elaborate controlled environment greenhouses in California. The glass roof was continually flooded by a stream of water to assist in temperature control and to diffuse light evenly. Air temperature and humidity were controlled independently. Movable trolleys or revolving tables were used further to even out variation between cultures and were designed so that the cultures could be sub-irrigated and controlled by time clocks. There were also dark rooms where artificial light was given in addition to temperature and humidity control.

Parker (1946) has described the constant environment rooms at Beltsville, Md (U.S.A. Department of Agriculture), used mainly in conjunction with sand cultures. Each room accommodates 58 2-gallon cultures. Humidity control ranges from 50-100% R.H. $\pm$ 10%. Lighting is entirely artificial and comprises 2,000 foot candles as arc light and 160 foot candles as incandescent lamplight. This mixture is stated to be important, but Parker (1946) and Withrow and Withrow (1947) have emphasised that no artificial illumination has yet equalled natural sunlight. Parker and Borthwick (1949) claimed, however, to have obtained growth of soya bean with artificial light to a degree equal or superior to that provided under glass with natural sunlight. The light source comprised 2,000 foot candles as carbon arc (from specially cored carbons) and an additional 160 foot candles as incandescent light. Parker and Borthwick (1949) and Withrow and Withrow (1947) both concluded that the spectrum of mercury vapour lighting is inefficient for plant growth, for which the carbon arc appears most suitable except for the considerable production of heat.

The effectiveness of a combination of two sources of light has already been noted. Parker and Borthwick (1950) found that carbon arc plus incandescent sources were greatly superior to fluorescent (4,500° white) plus incandescent sources. Withrow and Withrow (1947) found that mercury vapour plus incandescent showed no advantage over the inefficient mercury vapour source alone. Parker and Borthwick (1950) gave data on the deterioration in light to be expected when fluorescent lamps are used. A battery that initially gave 1,200 foot candles at 18 inches fell off to 1,000 foot candles after 300 hours and to 700 foot candles after 3,000 hours. The whole subject of artificial illumination is reviewed by Withrow and Withrow (1947) and by Parker and Borthwick (1949). There was some evidence that the nutrient solution composition required special consideration for the controlled environment experiments. Parks (private communication) stated that a water screen has also been included in the glasshouse units recently constructed at Beltsville.

Finally, Crocker *et al.* (1948) and Boyce Thompson Institute (Journal, Vol. 1) gave details of the range of environmental control available at the Institute. The greenhouses, which run East-West, include a temperature-controlled house with a range of limited spectral transmissions, temperature-humidity-controlled houses ; houses with external supplemental lighting, controlled temperature-humidity and carbon dioxide concentration, and light rooms with 25 tungsten filament and mercury vapour 1,000-watt lamps with water screens over an area 11 feet square, giving a minimum intensity of 400 foot candles compared with 10,000 foot candles for maximum June sunlight. It is pointed out, incidentally, that carbon dioxide may easily become limiting in a sealed " controlled " greenhouse.

Supplementary Light

The use of supplementary lighting for winter experiments has been studied by Low (1948) and Gelin and Burström (1949). The tubular fluorescent lamp appears to be the best all-round compromise in efficiency, growth response and low heating effect. A recent survey and extensive bibliography of artificial and supplementary illumination in horticulture was compiled by Brandon (1946).

Light Integrators

The measurement of total light reaching a particular region of a greenhouse over a period can be determined conveniently by simple light integrators. Various types based on photoelectric, photographic and photochemical means have been described respectively by Sprague and Williams (1941), Bayliss (1938) and Leighton and Forbes (1930). This last was based on the photocatalytic decomposition of oxalic acid by light in the presence of uranium ions. It is inexpensive, sensitive, simple to set up, precise and reliable. The total equipment only comprises a few similar glass tubes, a dark box for control tubes, pure standardised oxalic acid and potassium permanganate and a small quantity of uranium acetate. Unpublished experiments by the writer showed that oxalic acid concentrations of N/100, N/10 and N/1 were suitable for use to integrate light for periods of 1 day to 1 month during dull and fine weather. About 20 p.p.m. uranium should be present and accurately specified, as the amount of decomposition of oxalic acid is a function of the uranium concentration. The integrator could be calibrated against a standard light source of known characteristics. The oxalic acid-uranium acetate method was used by Tanada (1946) to record the light to which coffee trees were exposed in water culture experiments.

The Influence of the Biological Factors

The correct interpretation of results obtained in culture experiments is subject to the understanding that the cultural history, genetic constitution, nutrient status and natural variability of the experimental plants may all contribute to the observed results. Such biological factors are not readily controlled, but their influence can be examined and often minimised.

The Influence of the Seed on Growth

Any culture plant must have its origin in a spore, seed, cutting, tuber or similar body, which may contain substantial amounts of certain essential elements. It is also axiomatic that however small the " seed piece " (used in the broad sense) it will still contain at least a minute amount of the elements essential for the species. Some of these elements may not be sufficiently eliminated after several generations of culture plants to show whether omission is important, and this fact at present sets a limit to any attempt to declare that a potential micronutrient is not essential. It is only possible as Arnon and Stout (1939 a) have pointed out, to define an upper limit such that, if a potential micronutrient element is essential, it must be so in amounts less than this limit.

The elements present in seeds probably comprise a wide range outside those known at present to be essential, but data on this point are few. The concentrations of some nutrient elements in seeds are given by Beeson (1941) compiled from several sources. Examples of the range of concentrations of micronutrients recorded by Beeson are as follows in p.p.m. :—

From Beeson (1941)

Fe	Mn	Cu	Zn	B
Oat 7-350	Pea 4-12	Rice 2-4	Rice 3-30	Barley 2
Rice 12-76	Potato tuber 4-108	Maize 4-17	Beans various 18-56	Soya bean 6-29
Barley 14-350	Maize 5-19	Barley 6-41	Potato tubers 11-14	Potato tuber 2-16
Kentucky blue grass 390-460	Rye 32-157	Oat 4-51	Cotton 320	
	Kentucky blue grass 85-110	Cotton 54	Kentucky blue grass 360	
		Kentucky blue grass 60		

Piper (1944) found that the manganese and copper contents of rye and oat seeds may range about tenfold from 10 and 1 p.p.m. to 75 and 11 p.p.m. respectively. Erkama (1947) estimated that bean seeds contributed 2-3 micrograms each of manganese and copper per seed. Data on boron in seeds is given by Löhnis (1937) and Bertrand and Silberstein (1941); concentrations ranged from 30 p.p.m. in red clover seeds to 0·3 p.p.m. in oat and rye. Molybdenum content of seeds is variable. Bertrand (1939) found 0·6 p.p.m. in sugar beet, 0·9-1·7 p.p.m. in haricot and soya beans and 3·2-7·6 p.p.m. in pea and lotus. The embryo of beans contained 50 p.p.m. Mo as compared with only 2 p.p.m. in the cotyledons. Jensen and Betty (1943) found a range of 1-20 micrograms Mo per seed in clover types. The upper limit is near the threshold for total requirements of plants grown in culture solutions with added nitrogen. Hewitt (unpublished) found only about 0·1 p.p.m. Mo or ·0005 microgram per seed for a number of *Brassica* species.

Whilst it is likely that with a sufficiently careful technique deficiencies of all the known micronutrients can be produced in culture plants, the contribution of micronutrients made by the seed may be significant, especially where large-seeded plants with large reserves in the cotyledons are grown. The suggestion that nutrient-deficient seed should be first raised or selected for re-sowing or that non-embryonic parts should be excised has been made by Sommer (1926), Lipman (1938), Arnon and Stout (1939 a) and Smith and Bayliss (1942). For many purposes this does not appear necessary, but presumably it would accelerate the production of deficiency effects. Piper (1944) and (private communication) has pointed out that the difference in susceptibility of rye and oat to copper deficiency in water culture is due largely to the natural difference in the copper content of the seed of the two species. Virtanen (1949) reported that peas grown in tap water did not show copper deficiency, but that plants raised from the seeds of the first generation failed to reach maturity unless given extra copper. Wilson (1949) suggested that the molybdenum content of bean seed determined whether the plants would show scald. Hewitt and Bolle-Jones (unpublished) hastened the appearance of copper deficiency in beans by removal of the cotyledons on emergence.

It is not, however, correct to assume that the total mineral reserves present in a seed are always utilised under deficiency conditions. Buckner (1915) found that when seeds were sown in water a substantial proportion of the total calcium, magnesium, potassium and phosphorus was retained in the "exhausted" seed. The actual percentages retained in the seed ranged from a minimum of 20% K_2O in maize to a maximum of 54% CaO in bean. Softened sprouted potato tubers also retained the greater part of their reserves and it was concluded by Wallace and Hewitt (1947) that calcium in the tuber may be unavailable to the shoot in a calcium-deficient solution containing other requisite nutrients.

On the other hand, separation of the embryo from the cotyledons or endosperm or of the shoot from the remainder of a potato tuber may have other effects that will influence the experiment. Thus Virtanen and von Hausen (1949 a, b), Virtanen (1949) and von Hausen (1948) have shown that nitrate metabolism of some plants may be influenced in the early stages of growth by the supply of ascorbic acid available from the cotyledons and, if so, removal of these organs may materially affect the conclusions. Johnston (1924), Houghland (1947) showed that the growth and yields of potato plants were materially reduced if the tuber was removed from the shoot. Appleman (1918) and Hewitt and Jones (unpublished) found that increased weight of cut potato tubers left attached to the shoot correspondingly resulted in materially increased growth as dry weight of plants and tubers (grown in a recognised complete nutrient solution in the latter instance). These results may have a bearing on the controversial results obtained when organic supplements were included in nutrient solutions as already suggested, but in any case they indicate that removal of a part of the seed piece or any interference with the normal organisation of the young plant may have results possibly not directly connected with the mineral nutrient status.

The weight of unmutilated seeds may be important in precise experiments. Kidd and West (1918) reviewed extensive literature and concluded that there was good evidence to indicate that initial seed weight may influence final yields of pea, bean and cereal species by 10-50%. Schmidt (1921) (1923) also found that heavy seeds of soya bean, crimson clover

and buckwheat gave greater final yields in soil and in water cultures. Heaviest grade seeds were slightly inferior to medium heavy ones. Brenchley (1923) obtained similar results with pea and barley in water cultures. The superiority of plants raised from large seeds was most significant during early stages (short-term experiments), as smaller seeded ones tended to catch up over a longer period of growth. Colwell (1943) found no significant effect of seed weight on the accuracy of the sunflower method for estimation of boron. It may be concluded that for short-term and high-precision experiments some control of seed weight is advisable, and Brenchley (1914) (1916) and Clements (1928) used barley or pea seeds selected by weight between a narrow range.

Legumes present a special problem as, when nodulated, they are able to fix nitrogen. Virtanen (1933) found that nodulation was poor in water cultures but occurred freely in sand cultures. All legumes grown in sand culture at Long Ashton except lucerne have rapidly become nodulated spontaneously. The ability of legumes to obtain extraneous nitrogen from the atmosphere is complicated by the effect of molybdenum. This is essential both for nitrogen fixation and nitrate assimilation, but probably at much lower concentrations when nitrate is present. Experiments on nitrogen assimilation or metabolism with legumes must therefore take into account the effects of molybdenum supply. Water culture experiments with leguminous crops were reviewed by Bond (1950).

Variability of Culture Plants

Culture plants grown under apparently uniform conditions often exhibit variability in performance. This is due partly to genetic variation and partly to such factors as weight of seed and time and method of germination.

Genetic factors in maize have been shown by Hoffer (1926) to control absorption of iron and aluminium. Absorption and assimilation of phosphorus and nitrogen were found by Smith (1934), Lyness (1936), Hoener and De Turk (1938), and Burkholder and McVeigh (1940) also to be genetically influenced. Weiss (1943) found that efficiency of iron utilisation in soya bean is similarly controlled.

Rootstocks materially influenced the response of citrus and apple to lack of aeration and to nutrient deficiencies or absorption of nutrients in the experiments of Wallace (1924) (1928), Pearse (1937) (1940), and Haas (1946).

Methods of handling seeds prior to germination may influence subsequent development. Kidd and West (1918) concluded from a review of other work and their own, that the soaking of seed before germination materially affects subsequent growth. Different species react differently, most but not all being benefited by short periods of soaking ; the optimum time of soaking varies with species. Similar general conclusions were deduced by Tincker (1925).

Even when elaborate procedures to obtain uniform growth have been followed, individual variability between culture plants may be great. Davis (1921) found that individual water culture plants varied as much as 20-50% and recommended the use of the distribution curve as an aid to interpretation of results. Hibbard and Gershberg (1924) found that pure line Marquess wheat plants grown in water cultures had a dry weight variability of 5-6% at the 4 cm. height stage when handled in apparently identical manner. Davis and Hoagland (1928) found that the variability of wheat was only 3% after one month's growth in a closely-controlled environment. Chandler (1941) examined the seedling variability of cauliflower and other brassica crops when grown without boron. Some 7% of seedlings were able to develop first leaves whilst the remainder were not.

Design, Measurement and Interpretation of Experiments

It is evident that culture experiments are liable to suffer from numerous sources of error. Even if elaborate methods are used in attempts to avoid or reduce many of them, further precision may be achieved by attention to the design and layout of the experiment, so that their effects are either measured as " error " or distributed as evenly as possible between replicates or treatments.

Arrangement

Four types of arrangement of cultures are possible, namely, systematic, revolving table, frequent change of relative position, and randomised block and factorial arrangements. Systematic layouts are permissible when comparative visual studies are the main object, but even then variations in lighting or other factors may introduce error. They are convenient when a number of treatments are involved in which no trace of cross-contamination can be allowed. Minor environmental differences appear unimportant where gross effects on visual behaviour are concerned.

The revolving table was introduced by Shive (1915 b) in an attempt to eliminate errors due to uneven environment and achieved limited popularity for a number of years. This device was used by Livingstone and Tottingham (1918), Johnston (1920) (1921), Davis (1921), Tarr and Noble (1922), Hibbard and Gerschberg (1924), Tottingham (1926) and Clements (1928). Speeds of turning ranged from one revolution in 2 minutes, Johnston (1921) to one in 4 days, Tarr and Noble (1922). Recently they have been used also by Eaton (1941 a) and Went (1943 b) in combination with sub-irrigation with a mercury seal to transmit the necessary air pressure. In general, however, such arrangements are only suited to a few cultures owing to the limitation in size and the mechanical difficulties involved.

Frequent re-arrangement of cultures in new relative positions has been adopted in a few instances by McCall (1916 a), Shive and Martin (1918 a), Davis (1921), Woodman (1939 b), Machlis (1941) and Mullison and Mullison (1942). Bond (1950) combined frequent re-arrangement with randomisation. The method of re-arrangement shares the inequalities of environment between the cultures, provided that these inequalities do not vary relatively when the weather changes. The method is time-consuming, disturbs the cultures and has the disadvantage also that the layout cannot be " memorised " as is quickly possible even with a complex randomised experiment. Easily moved small tables, as used by Cook and Millar (1946) are helpful here.

None of the above methods of arranging cultures allows of any quantitative measure of the precision and error of the experimental data or the estimation of their significance, i.e. the probability that they are valid and not coincidental or due to unsuspected environmental, biological or other external interference. This precision can only be obtained by use of a recognised statistical procedure applied to a layout correctly designed to eliminate or balance error and to extract the maximum amount of required information. Numerous papers dealing with possible ways of applying statistical procedures to culture experiments have been published. No competent criticism or appreciation of these can be offered here. The Agricultural Research Statistical Service established at Rothamsted under the direction of Dr. F. Yates, F.R.S., is concerned in the advice, layout and analysis of officially sponsored experiments in the United Kingdom.

One or two comments on the statistical approach are, however, appropriate. Space available for culture experiments is usually limited either by physical considerations of space, or by water supply or cost of equipment, or the abilities of the experimenters to cope with the work. Where complex problems of nutrient relationships are under investigation, the factorial approach is usually the most efficient, especially for limited replication. Owing, however, to the limitations just mentioned and the frequent importance of the high-order interactions, the opportunities for confounding are limited.

The layout of culture experiments should take into account the characteristics of the greenhouse space. These include width, number and direction of benches, likelihood of draught, distribution of ventilators, heating pipes and light and shade. Special attention has been given in papers by LeClerg (1935) and more particularly by Wadleigh and Tharp (1940), Youden (1940), and Cox and Cochran (1946) to the accommodation of these factors. Split plots, irregular or incomplete blocks and other devices adapted for use in greenhouses with inconvenient arrangement of benching or with large sand beds have been described. Wadleigh and Tharp (1940) adopted an attitude seldom admitted amongst statisticians when they suggested that " systematic procedures for elimination of (costly and inadmissible) mistakes in watering are a superior need to the use of a theoretically ideal random layout."

This qualification would appear less important, however, if a suitable coding method for quickly identifying different treatments without chance of confusion is selected (as described in Part Two) and if sufficient space is left between cultures where even a stray drip or splash is totally unallowable, as for example in studies on molybdenum nutrition. The problem of replication in greenhouse experiments is discussed by Cox and Cochran (1946) and by Woodman (1946). The greater the number of intermediate levels in a series of treatments, the greater is the required replication. Two standard works on the subject of experimental design and layout are those of Yates (1937) and Fisher (1942).

Design

The design of culture experiments in relation to the amount of information that can be obtained has been discussed by Richards (1944). There is no means at present whereby the independent effects of cations and anions can be properly estimated, as change in the concentration of any given ions must be accompanied by either corresponding change for ion of opposite sign or complementary change for other ions of the same sign, or both. Gregory (1928 b) attempted to solve this problem by assuming that all ions affect growth independently, in either positive or negative manner and that the overall effect is probably a summation, product or still more complex function of the individual concentrations. Gregory ingeniously used data of an experiment by Johnston (1924) in which all six of the possible 3-salt combinations had been used, to test the hypothesis. Yields recorded by Johnston were calculated as the per cent. of the mean yield and equated to the ionic proportions of the elements present in each of the six nutrient types for all the combinations producing an osmotic pressure of 1 atmosphere, when the amount of each salt was varied in such a manner that it provided between 1 and 6 eighths of the total osmotic pressure. 123 equations were derived and the method of least squares was used to deduce the fraction of yield (either positive or negative) due to unit concentration of each ion. The fact that the total ionic concentration varied in each solution, in order to maintain the constant ratio and osmotic pressure, required the introduction of correction factors. These were derived from a series of approximations based on mean values obtained after re-grouping the equations to eliminate each ion in turn, and then substituting the value found in the next group and so on. Results were plotted against yield and the effect of increments of a specific salt were shown as curves of various types. The analysis was a classical example of an attempt to use purely mathematical means of deducing the separate effects of normally paired ions. Calculated values agreed well with experimental ones. Increments of Ca^{++}, Mg^{++} and NO_3' had uniformly positive effects on yield ; K^+ was positive up to 4/8 and PO_4''' uniformly negative. $MgSO_4$ was indifferent ; KH_2PO_4 was slightly positive at low concentrations and then slightly negative. $Mg(NO_3)$ had a similar but greater effect, whilst KNO_3 and $Ca(NO_3)_2$ were uniformly positive in effect on yield. The relative merits of the triangular and pyramidal designs introduced to plant physiology by Schreiner and Skinner (1910) and widely employed by Tottingham (1914), Shive (1915 a), Clements (1928) and others referred to in the Section on nutrient composition, and of the more modern factorial approaches have been critically reviewed by Richards (1944). The latter permit of statistical analysis whereas the triangular methods do not, and interactions are confounded. Possible improvements in the use of triangular procedures and their extension to cubes, prisms and pyramids were also suggested by Richards (1944).

Useful, though restricted, information on ion interaction effects and responses to individual ions can be obtained by the device already referred to, in which the triangle method is combined with a constant summation of either anions or cations, whilst ions of opposite sign are varied. This method was introduced by Beckenbach *et al.* (1936) and used also by Wadleigh (1939), Hamner (1940 a), Mullison (1941) and Mullison and Mullison (1942). Essentially the method consists in arranging that a group of 3 (triangular system) or more (polyhedral system) pairs of ion variables are distributed in two groups. In one group, the anion set, the cations are all supplied at constant concentrations, but the anions are varied in the usual differential manner. Thus the concentrations of Ca^{++}, Mg^{++} and K^{++} ions

are held constant whilst NO_3', PO_4''' and SO_4'' ions are varied differentially with the same total concentration as equivalents. In the second set the anion concentrations are held constant while those of the cations are varied. The change in concentrations of one set of ions, while those of opposite sign are unvaried, is achieved by inclusion of as many salts for *each* ion as there are ions to be varied. Thus for a 3-dimensional (triangular) system comprising NO_3', PO_4''' and SO_4'' *each* of these ions would be supplied in combination with Ca^{++}, Mg^{++} and K^+. It is then easy to arrange that the total concentrations of either set may vary whilst the sums of each of those of the complementary set are held constant. Statistical analysis can be applied to the results and limited deductions as to the effects of individual ions can be made.

An interesting example of the application of *Graeco-Latin* squares to the layout of an experiment where equipment was restricted was provided by the work of Woodford and Gregory (1948). In their experiments on aeration and nutrient absorption already described, only two culture vessels were available. There were 16 treatments in the factorial experiment and 4 replications were used. Only two treatments could be given successively to the same batch of plants and thus only 4 treatments were possible daily. Each block selected at random was thus completed in 4 days and the order equivalent to arrangement of treatments was determined beforehand from the " layout " of the 4 *Graeco-Latin* squares. Analysis of variance showed the procedure to be justified.

The Measurement of Responses of Culture Plants

Growth is most commonly used to assess the effects of culture treatments. Growth has been measured by various means including total yields of fresh or dry weight of parts or the whole of the plants, rate measurements such as " nett assimilation rate " discussed recently by Heath and Gregory (1938), Williams (1946) and Watson (1947), weight at unit intervals of time, height, leaf area, tiller number, transpiration, or carbon dioxide assimilation, measured by Gregory and Richards (1929).

For many purposes total dry weight yield (subdivided if desired for different morphological parts) provides a useful index of the overall course of the experiment. If it is suspected that the response has included a differential delaying or acceleration effect on growth, later evened out due to the duration of the experiment, the use of a growth rate determination may be required and may always provide valuable additional information. Fresh weight determinations, although less reliable owing to the likelihood of appreciable variations in moisture content, are helpful if oven space is limited. They may serve to indicate gross differences and are also convenient where tubers or storage root organs are used to estimate growth. Total transpiration does not appear to possess any particular advantage as a criterion of growth, except that it can be measured independently (though perhaps not easily with accuracy) without interference with the plants. Climatic conditions, however, greatly affect transpiration and this criterion can therefore only be used for closely controlled simultaneous and *not* sequential comparisons.

The function now termed " nett assimilation rate " is based on the growth formulae suggested by Gregory (1917) and by Briggs *et al.* (1920) and West *et al.* (1920). It provides a measure of the growth of the plant in terms mainly of absolute carbon assimilation (dry weight) per unit mean leaf area in unit time. It is therefore independent of assumptions based on exponential or proportional growth formulae, and is in fact a measure of assimilative capacity of the foliage. In exceptional instances, however, the " nett assimilation rate " may be expected to be misleading. Thus Stout and Meagher (1948) reported that molybdenum-deficient tomato plants accumulated up to 14% of dry weight as *unassimilated* nitrate which was reduced in less than 48 hours after injection by molybdenum. It is also necessary to recognise that nitrogen content is an appreciable fraction of total dry matter and that nitrogen uptake may not necessarily show the same relation to leaf area as carbon assimilation. Williams (1946) suggested that although dry weight (in leaves) might be a satisfactory determination for use during early growth, leaf protein gave a better indication of growth, especially with limited nitrogen supply.

Portsmouth (1949) described a modified half-leaf method of interest in relation to measurement of assimilation in culture experiments. It comprised the determination of leaf areas by a rapid tracing and blue print technique and of fresh and dry weight measurements on a sensitive torsion balance. The method accounted for asymmetry of the leaflets and only one-half to one-fifth of the replication was required to give the same accuracy as in the Sachs's half-leaf method, an obvious advantage where only limited culture material is available. Vickery *et al.* (1949) compared three sampling methods for determination of leaf composition as affected by culture treatments. A systematic latin square gave the greatest precision and required fewer replicates than either an opposite leaf method or a leaf size method.

The measurement of toxicity is most readily accomplished by measurement of depression of growth. Brenchley (1910) (1914), and Machlis (1941) used the first sign of any measurable growth depression as a criterion for comparative toxicity experiments. Gile (1938) suggested that comparisons should be made for concentration of toxic agents producing a 50% depression in growth as this is a more easily recognisable stage. Further information on toxicity tolerance (and sub-toxic-stimulatory effects) is obtained by plotting growth depression or stimulation for a suitably graded exponential range of concentrations. Strict quantitative treatment of the results is obviously related to the problems of bio-assay and cannot be described here. There may be a wide range between concentrations just perceptibly toxic and those that cause a 50% reduction of growth as shown by Davies (1941).

Other criteria may sometimes be necessary, e.g. measurement of chlorosis or greenness either by use of a colour standard or by actual determination of chlorophyll content which can be easily performed by a routine method on small samples, or by recording morphological characters such as leaf angle, length of internodes or number of tillers in relation to time.

Visual observations have an important function entirely distinct from quantitative determination of growth. Visual symptoms are manifestations of the hitherto qualitative and physiological responses of the plant illustrated in a manner that cannot yet be replaced by growth measurements, determination of leaf pigments or other chemical data. Accurate and detailed visual studies indicate the morphological distribution and possible nature of underlying physiological abnormalities, knowledge of which is necessary to subsequent more detailed, anatomical and biochemical studies.

The Interpretation of Data obtained in Culture Experiments

It will be apparent from what has been said in the Sections dealing with the biological characteristics and influence of environment on culture plants, that the results of experiments may depend greatly on such factors. Strictly speaking, therefore, the data obtained with culture plants can be interpreted only with reference to a specific set of environmental conditions. These are likely also to have varied during the course of the experiment and it may not always be possible even to specify them. For these reasons the interpretation of results obtained with culture plants and their more general application to field problems and growth processes must be made with caution. In some instances, however, it is reasonable to infer that environmental factors other than those intentionally varied, do not exercise a critical influence on results and conclusions of a more general nature are justified. It is particularly desirable, even in such instances, to confirm results and conclusions obtained in culture experiments by field trials where possible or relevant. Water cultures, and in some respects also sand cultures, can never completely reproduce the effects of natural soil habitat, and it is difficult to simulate such aspects as oxygen and carbon dioxide concentration, buffer capacity, water tension and exchange complex, especially the little understood soil organic matter. Size of container alone may outweigh all other effects in some instances, and no correspondence between nutrient concentration and rate per acre is possible.

The special value of sand and water culture methods depends more on the fact that culture methods can eliminate and then partly reintroduce under controlled conditions selected combinations of the complex of factors comprising a soil environment. Culture methods permit the study of interactions of deficiencies and excesses at accurately determined

levels, assist in the determination of probable relative nutrient requirements, and also allow the effects of total omission of nutrients or possible nutrients to be observed, if necessary, in relation to light and temperature. These problems cannot be studied completely in any natural soil at present owing to the undetermined complexity of the many interrelating factors present and the almost inevitable presence of positive, but not readily evaluated, concentrations of many microelements. For the same reason interpretation of results obtained in culture must be qualified by the same degree of uncertainty when the conclusions are applied to plants growing in the field. In addition to such causes of uncertainty, it must always be realised that field conditions differ from those associated with culture plants in other important respects. An outstanding distinction is the extreme nature of the precautions needed to demonstrate certain micronutrient deficiencies in culture compared with their often widespread and acute occurrence in the field. Factors of availability are often the cause of the latter, but do not influence the former. Others include exposure to wind, rain and drought, competition from weeds and animal destruction, and the great variation in soil profile, watertable and composition. These contribute in unknown degree to the response of plants growing under adverse nutritional circumstances and may greatly accentuate the severity of their ill-effects as compared with plants grown in culture. A useful discussion of some of these problems as they affect the comparison of field and of greenhouse trials with soils is given by Cook and Millar (1946).

The interpretation of experiments to study the absorption, translocation and role of mineral elements and their interrelationships must also be qualified by the probability that the more widely any of the treatments used departs from the normal environment (mineral or climatic), the greater may be the element of uncertainty attached to the inference of conclusions. As, however, knowledge concerning the effect of an element in the promotion of normal growth and metabolism can usually only be obtained by comparison with abnormal material, this limitation seems almost inevitable. The use of carefully graded factorial treatments just sufficient to produce significant differences in composition or metabolism would seem to represent the best compromise to adopt in the solution of these problems.

Part II

THE LONG ASHTON TECHNIQUE FOR LARGE SCALE POT SAND CULTURES

OBJECTS AND REQUIREMENTS OF TECHNIQUE

The methods described in this part were based on the original technique described by Professor T. Wallace (1933) at Long Ashton for the growth of fruit trees in sand cultures and have been developed and extended during the last nine years with especial emphasis on the use of agricultural and horticultural crop plants as culture material. Much of the work undertaken centred on the use of the visual methods developed for the diagnosis of nutritional disorders and was later adopted as an approach to the study of the physiology and biochemistry of crop nutrition under controlled conditions. The technique has been evolved mainly in relation to extensive surveys of the responses of numerous and diverse crop plants to several variations and combinations of nutritional factors. The technique thus has had to fulfil the following requirements :—

(a) All stages of preparation of materials and subsequent treatment of cultures must be applicable to large numbers of cultures and large quantities of materials without appreciable loss of efficiency.

(b) As future developments of the work might involve unforeseen requirements, the technique must be adaptable and precludes the use of elaborate equipment and automatic methods of nutrient regulation in the initial stages of development.

(c) Work on a large scale requires the service of a number of junior assistants and methods for the preparation and application of nutrients and care of the cultures must be simple. The possible effects of breakdown in elaborate equipment used on a large scale also emphasises the value of simple methods.

(d) The large quantities of basic experimental materials, e.g. containers, water, etc., impose the need for reasonable economy in refinements relative to the special objectives, e.g. elimination of macro- or micronutrients from materials.

(e) The technique must be applicable to the study of micronutrient deficiencies, which, at Long Ashton, have so far included those of iron, manganese, boron and molybdenum, and lately copper and zinc.

The relative merits of sand and water culture methods have already been outlined. Sand cultures were considered on the whole to be the more satisfactory for the growth of a wide range of plants and the scope and purposes of the work.

The technique as it stands represents a stage of development ; partial or complete solutions to most of the problems involved have been achieved. The technique, however, is not static, and innovations are continually being introduced. The existing methods are, nevertheless, reproducible and adequate for the work that has been undertaken so far.

BASIC MATERIALS USED

The basic materials suitable for use in this technique depend upon the work to be undertaken. The containers may be horticultural clay pots, 5-litre pyrex glass beakers or other pyrex glass containers provided with a central drainage hole or containers made of polythene. The sand is a natural silica sand obtained from Leighton Buzzard in Bedfordshire; an alternative source of pure white sand from Loch Aline in Scotland has also been used. Water is obtained mainly as rain-water from the roof of the main greenhouse which shelters the cultures : it is used directly or purified by the Permutit ion-exchange method. Distilled water may be required for some purposes where a smaller scale is permissible, and tap water may suffice for others.

The nutrient solutions are made up either from recrystallised (laboratory) grade reagents or from analytical reagent grade compounds which may require still further purification. Experimental plants are either raised in John Innes' greenhouse compost or in pure sand or sown direct in the sand in the containers. The choice, preparation and value of these materials is discussed in detail in the following sections arranged according to the particular material concerned.

CONTAINERS

The Clay Pot

The container used most frequently is the 10-inch clay pot, preferably with a single drainage hole. This holds 9 kilograms of sand. The pots are made waterproof and unreactive by painting or spraying the interior with 3-5 coats of pure bitumen solution. The grade is *Bituros Solution* obtained from Messrs. Wailes-Dove, Hepburn, Co. Durham. This material has the following analysis :—

Ash % 0·142	Fe	Mn	Cu	Zn	Mo
Analysis % in Ash	2·5	Not detected	1·7	0·085	0·017
p.p.m. in original	30	—	20	1	0·2

The use of paint or some protective coating is especially necessary to avoid contamination from boron and calcium ; magnesium and potassium are also readily extracted in water from the clay.

Experience has shown that it is better to apply the paint to dry untreated pots. Pots previously waxed are not satisfactory as the wax is liable to "sweat" through the paint coating. It is important to continue the painting through the drainage hole and on the outside of the base and over the rim at the top. The periphery of the drainage hole should be smoothed down, if necessary, and the pot brushed free of all dust before painting. Pots that are cracked or have large irregular drainage holes are unsuitable and should be discarded. The surface should be inspected for lime nodules which may occur beneath a thin layer of pottery that may eventually come away and break the paint film. Pots with large or numerous nodules should not be used.

The pots may be warmed to feel hot to the hand before applying the first coat of paint to assist in penetration. The succeeding coats may be applied as soon as the previous one is dry to handle (two days usually suffice), and about ten days' interval should elapse before filling to allow all volatile constituents to evaporate.

At the end of the season the pots are dried after emptying, the residual sand is brushed out with stiff bristles and the pots repainted with three coats of paint. They thus become progressively less reactive. Pots are used again for similar nutrient treatments to minimise effects of previous history.

The sand is retained in the pot by a thin felt of glass wool placed over the drainage hole and covered by a 2·5 inch diameter watch glass, convex side upwards. The glass wool is cleaned by extraction with constant boiling hydrochloric acid in a large soxhlet apparatus and rinsed in distilled water. Watch glasses are boiled in hydrochloric acid. No instances of roots penetrating this filter have occurred to date and the method provides free drainage without loss of sand.

Examination of the paint surface after a season's use generally shows that little or no interaction between the roots and the pot has taken place and root matting does not occur appreciably, whilst roots that have been in contact with the paint surface appear white and healthy.

Clay pots painted in the manner described are suitable for the study of all problems involving macronutrient elements and toxicity problems. They have also been used successfully for the study of deficiencies of iron, manganese and boron. Experiments show that they are not suitable for work with molybdenum deficiency. It is unlikely that they would be suitable for copper deficiency since the paint contains this element in appreciable amounts. The period for which the paint is effective has not been determined, but would appear to be at least six to nine months.

Before the use of bitumen paint other methods were tried to prevent interaction between the clay pot and the plants. These included waxing treatments and application of synthetic resin varnishes and lacquers. They are noted in Part One.

Glass Containers

These are of two types. For many crops five litre, tall-form pyrex or hysil glass beakers without lip, provided with a central three-quarters inch drainage hole (by arrangement with the makers) are suitable. Their price is about 15 shillings each, which compares favourably with 10-20 shillings for a glazed one-gallon container. The other type of glass container is the pyrex glass urn liner* available in capacities between 1 and 6 gallons. These vessels have a domed, concave bottom and a central, ground drainage hole. The top is flared and the walls are about 0·2 inch thick. The prices range from 10 shillings for the 1-gallon size to £2 5s. for the 6-gallon size.

The glass pots are cleaned as follows : They are first washed in hot water with a detergent and then exposed to a spray of " steam " from constant-boiling hydrochloric acid followed by careful rinsing with glass distilled water or are steamed after immersion in strong acid and then rinsed.

Sand is retained by a watch glass and glass wool as for the clay pots. A five-litre beaker holds 7 kilograms of sand. The absence of a lip is desirable as such pots are mechanically stronger and hold a greater quantity of solution without overflowing. The pots are supported on wooden stands measuring 8 inches square with a 1-inch deep circular " well " to hold the beaker or urn liner, and a 2-inch central hole. The stands are waxed in boiling paraffin wax. A dowel rod fixed in a hole in the stand may be used for supporting tall or weak-stemmed plants. Algal growth and light are excluded by wrapping a double layer of heavy brown paper around the pots. Glass beakers have not the same strength as clay or glaze and more care in handling and carrying is needed. Root growth rarely causes breakage by expansion ; casualties of this nature have only been about 2-3 annually. Pyrex glass containers are suitable for the study of copper, zinc and molybdenum deficiency and may also be used for work with iron and manganese deficiencies. Their composition may render them unsatisfactory for work with boron deficiency, especially at high pH values.

Plastic Containers

Self-supporting containers made from cellulose acetate and polythene sheet have been tested for use for some micronutrient deficiency studies. Cellulose acetate, although initially promising as a material from which to make containers, was abandoned for two reasons. It was found to contain some toxic plasticising compound which caused severe injury to many seedlings and had to be removed by a preliminary hot water treatment. The containers, which had to be made by cementing sheet together, became brittle and deformed after use or storage and the seams tended to come apart.

Self-supporting polythene containers shaped like 10-inch clay pots have been supplied to specifications at the price of 22 shillings each. These have been tested for suitability for work with iron, manganese and boron deficiencies and, in a preliminary trial, for molybdenum

* Obtainable from Messrs. James Jobling, Weir Glass Works, Sunderland.

deficiency for which they appear satisfactory. No toxic effects were produced with these containers. Attempts to spray melted polythene on to clay pots proved difficult and the results were unsatisfactory owing to splitting and breakdown of the film thus produced.

Glazed Containers

Standard, one-gallon, glazed jars* have been tested for use in iron, manganese and boron studies. They appear satisfactory for work with manganese and boron deficiencies, but are unsuitable for iron deficiency, as test plants failed to produce any symptoms of lack of iron when grown in these containers. Other types of glazed containers have been tested for traces of soluble micronutrients in the glaze and revealed great differences in composition. A semi-porcelain type† was outstanding and yielded only low concentrations of iron, manganese, zinc, boron and molybdenum.

SAND

Source and Grade

The sand mainly used in experiments with vegetables and fruit trees is a relatively pure silica sand obtained from Leighton Buzzard, Bedfordshire.‡

The choice of sand was determined by particle size and by the nature of the major impurities present, details of which have been given in Part One. This sand allows vigorous growth of a wide range of fruit and vegetable crops from early development to maturity.

An alternative source of sand from Loch Aline, Scotland, has been tested recently, as noted in Part One. The relative freedom of this sand from iron and the evidence of its ability to support vigorous growth suggest that it may be used with advantage for experiments on iron nutrition. The particle size, although finer than that of the Leighton Buzzard sand, is satisfactory.

Treatment of Sand before Use

The sand is obtained as " water-washed " and this treatment at the quarries serves to remove silt and clay. For many experiments with macronutrient elements, leaching with water is sufficient treatment before use. For precise experiments with these elements and the study of any problems connected with calcium, further treatment is necessary. This treatment comprises saturating the sand with cold 3% hydrochloric acid and leaving the saturated sand in contact with the acid for a week before leaching. The sand is leached initially with water, and then twice daily with the appropriate type of nutrient solution until the leachate has the same pH value as that of the nutrient solution and shows no change after remaining in contact with the sand for 24 hours. Sand may be leached in large clay (bitumen-painted) pots or 25-gallon glazed vats. Bromo cresol green is a suitable indicator for the purpose. Leaching sand treated as described may take two weeks or more but should on no account be omitted. This leaching is necessary to displace adsorbed hydrogen ions which would otherwise cause severe injury to plants.

Sand required for experiments with micronutrient problems must be purified more rigorously. The purification involves first a mechanical removal of the coarser, more heavily contaminated particles and then an acid extraction in a specially designed apparatus. The first procedure eliminates a significant fraction of the micronutrient impurities present, as these are often associated with the coarse fragments of iron sandstone and conglomerate present in the sand. It is effected by sieving the air-dried sand on a 24-mesh sieve of pure copper or stainless steel. A continuous rotary sieve is at present undergoing trials. Rotation at 70-80 p.p.m. permits complete separation when a cylindrical sieve 4 feet long by 10 inches in diameter is used. The sieve is inclined at an angle of about 15° from the horizontal and sand is fed into it by hand. Power is obtained from a $\frac{1}{3}$-h.p. capacitator motor with a counter-shaft and a belt drive on to a cycle wheel. Sand required for copper and zinc deficiencies

* Messrs. Royal Doulton Lambeth Potteries. Standard cream glazed coffee jar.
† Messrs. Hathernware, Loughborough, Leicester. Semi-porcelain T.K. G69 white glaze.
‡ Messrs. Arnolds Quarries. Double Arches Pit No. 21 washed.

is sieved through stainless steel gauzes. The extent of copper contamination from a copper gauze may be judged from the data on sieved sands shown in Table 7.

The acid treatment is required to remove the bulk of the micronutrient impurities present. It was essential in undertaking large-scale experiments to develop a suitable and economical method for the efficient treatment of the several tons of sand needed for a season's work. Between 1944 and 1946 sand was purified in batches of 40-50 lbs. by blowing steam at 20 lb. per sq. in. pressure into sand saturated with hydrochloric acid in a wooden 9-gallon barrel (previously cleaned with boiling acid). The initial acid concentration was 9-10%. Hydrochloric acid was used alone in 1944, but 0·5% oxalic acid was included subsequently as this reagent has been used in methods for purifying sand in glass making to increase the solubility of iron compounds by Adams (1935) and Curtin and Parker (1940). The value of the oxalic acid may be judged from the observation that cold dilute hydrochloric acid and oxalic acid will whiten sand whilst hydrochloric acid alone will not. Tests have shown that 15-18% hydrochloric acid is more effective and the oxalic acid concentration is now 1%.

The crude equipment has been replaced by the apparatus shown in Figures 7 A & B. It was developed after experiments with glass laboratory-scale and large-scale models, and is constructed in " Keebush " acid-resisting material from the following standard parts* : The smaller of the two models has been used for 4 years. The cylindrical vessel is of 18 gallon capacity and has a " blocked-in " conical bottom with a central aperture. Through this aperture a central tube projects nearly to the top of the vessel. The tube has an internal bore of $\frac{3}{4}$-inch and two tiers of alternating $\frac{1}{2}$-inch holes are arranged around the tube as close as possible to the base of the vessel, to permit the intake of the sand-acid mixture. A steam jet $\frac{10}{64}$-inch internal bore projects through the central tube for $1\frac{3}{4}$ inches beyond the upper limit of the holes. The jet is held in position by fibre washers against a collar which fits in the enlarged bore of the tube in the region of the flange connection. The central tube itself is held to the base of the vessel by a steel plate bolted against a collar ring attached to the tube and is sealed to the base of the vessel by a rubber gasket. The sand and acid are drawn into the central tube by " suction pump " action of the steam jet and transported up the tube by the force of the steam as it issues from the jet under a pressure of 12-15 lb. per sq. inch. The dimensions of steam jet diameter and position, arrangement of holes and steam pressure which produce maximum rate of circulation are all closely interrelated. They were determined in extensive trials where the descent of an annular plate was used to measure transport. The measurement of transport of sand in the larger model was achieved by the use of a removable catch-pot which was weighed when about 20 pounds of sand had been collected in a period of 30-40 seconds.† The steam is led to the jet by a hosepipe connection attached to a radiused " T " piece (not shown) aligned in such a manner that any acid which drips out of the jet does not run into the steam line but collects below the radiused junction and can be drained off through a Kestner-Saunders valve. Draining valves are also located as near as possible to the base of the vessel to facilitate the replacement of " spent " acid during the treatment. The sand is distributed over the untreated sand which is thus displaced downwards ; circulation is therefore continuous and automatic. This model can circulate a 250-pound load of sand at the rate of over 400 pounds an hour, which means that five complete circulations are completed in three hours.

A larger model has been designed and tested to determine its ability to transport sand. A provisional specification includes the following details : The capacity is a little over 600 pounds of sand in each batch, and circulation is not less than 1,500 pounds an hour ; actual determinations have shown that the rate may exceed 1,800 pounds an hour. It is thus evident that the time for complete circulation of a batch in the larger model is not greater than that in the smaller one and may be less. The central tube has a 1-inch or $1\frac{1}{4}$-inch bore. The assembly of the jet and central tube has been redesigned for rapid dismantling and replacement. This is achieved by the use of special annular hollow screwed plugs developed by the Kestner

* These are manufactured by Kestner Evaporator and Engineering Co., Ltd., London.

† This work was carried out with the collaboration of Mr. E. W. Jones, and Mr. E. J. Winter whose share is gratefully acknowledged.

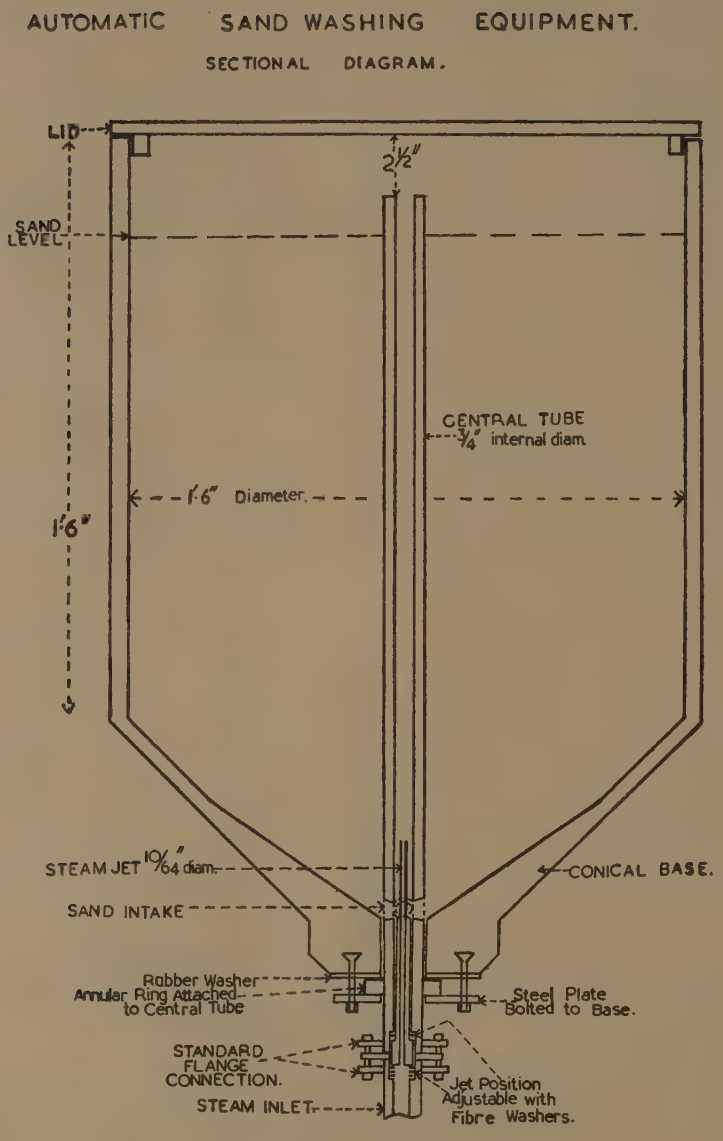


FIGURE 7A

Diagrammatic assembly of "Keebush" Sand Purification Unit, showing arrangement of jet, central tube and sand intake (Hewitt 1947a).

Engineering Company. These hold the steam inlet, jet and central tube together in the base of the machine. All metal flanges and clips have thus been eliminated. Spent acid is drained through a sintered filter. A large port, wide enough to take a hand, is located near the base of the cone to unload the sand direct into leaching vats. The lid is held inside the upward extension of the walls of the vessel to retain spray and drips. A funnel for addition of acid and a pyrex glass drop-in inspection port are also included. The overall height of the apparatus is approximately 6 feet. The original diameter of 18 inches has been retained as it was thought that with a diameter of 22 inches (the next standard mould) circulation of the sand might not occur efficiently near the periphery.

The important advantages of this apparatus are as follows : Little attention is needed during a run other than an occasional check on steam pressure. The weight of sand treated is large and the shape of the vessel and the circulation reduces the possibility of untreated " pockets." Sand grains are simultaneously heated and agitated in contact with the acid. A given weight (say 250 lbs.) of sand that was treated in a day's work previously in small batches for one hour each can be treated for a much longer period for the same throughput. Condensation is relatively less over a long period for a large bulk of sand, and acid concentration is conserved proportionately. Moreover, greater uniformity in treatment is possible. A general view of the sand treatment apparatus is shown in Plate D.

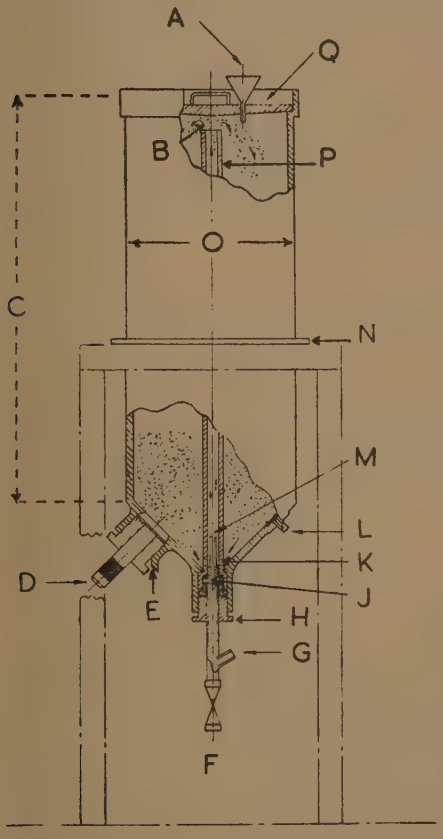


FIGURE 7B

New Kestner " Keebush " Sand Digester

A = Acid filling.
B = Sand circulation shown by small arrows.
C = 2'-4' as required.
D = Sand outlet plug held by hollow plug.
E = Lip for pouring.
F = Drain, and Kestner-Saunders valve.
G = Steam connection.
H = " Keebush " hollow screwed plug.
J = Jet packing and gaskets.
K = Sand intake.
L = Acid outlet through 10 cm. glass sinter.
M = Jet 10/64" i.d. in base of central tube.
N = Moulded ring supporting apparatus on wood stand.
O = 18" internal diameter.
P = 1" central tube.
Q = Removable cover with pyrex glass inspection port.

Routine Use of Sand Purification Equipment

The method recommended for routine operation of the equipment is given below. The duration of treatment and number of acid changes may be modified as the result of experiment with different types of sand, but the data given here are those relating to the use of the Bedfordshire sand for the smaller size model.

Typical Run : Part I

8.55 a.m.	...	Boiler* is switched on (steam raised in 5-7 minutes). Steam is turned on to 1-2 lb. per sq. in. pressure ; 2 jars of prepared acid mixture are added to vessel.
9.0 a.m.	...	Steam is turned up to 12-15 lb. per sq. in. ; run commenced.
9.0—12 noon	...	10 litres HCl are placed in stone jar, 310 g. oxalic acid dissolved in 1 litre boiling water and added. Sand of previous run (contained in glazed vat) is leached through with 20 L. distilled water or demineralised rain water and allowed to drain. Pots are brought to site, and wiped on inside with cotton wool to remove dust. Drainage hole of each pot is covered by a thin pad of glass wool, previously extracted with 20% hydrochloric acid for 6 hours, and a watch glass. Ladling jugs are rinsed with tap water inside and outside before use. Any scum on surface of sand in vat is skimmed off before ladling. *First portion of sand is placed carefully onto watch glass in each pot.* Experiment number is chalked on each pot. Pots are placed on benches and covered with brown cardboard.
12 noon ...	...	Drainage valve of machine is opened and acid allowed to drain away. Steam is kept at 12-15 lb. pressure. When drained, steam is turned down to 1 lb. pressure, tap is left open and prepared acid mixture is added. Any residual oxalic crystals are dissolved with boiling water (1-2 L.). Drainage is continued until the acid can all be admitted, leaving an inch between acid level and lid.

Part II

12.40 p.m.	...	Drainage tap is closed. Steam pressure is turned up to 12-15 lb. pressure and run continued for 3-4 hours.
12.40—4.0 p.m.	...	To *each* of two clean stone jars 5 L. hydrochloric acid, 155 g. oxalic and 12 L. of boiling water are added. Sand is brought over for reloading for next run. Glazed leaching vat is washed out and bung inserted tightly into outlet of vat. Drainage hole on inside of vat is plugged with acid-washed glass wool. About 10 L. $Ca(NO_3)_2$ appropriately purified is siphoned in readiness for leaching into a stone jar kept solely for that purpose.
4.0 p.m.	...	Steam is turned down to 1 lb. pressure ; acid is drained off. Sand is ladled into vat with thoroughly cleaned jugs.
4.15 p.m.	...	When machine is empty, steam is increased to 10 lb. to clear jet. Pad of glass wool over inside aperture of drainage valve is replaced (unnecessary with sintered disc). Steam is turned off. Jet is sealed by inserting a glass tube down central tube between outside of jet and inside of central tube or alternatively by a small rubber bung inserted in the glass tube and allowed to rest on top of jet. Vessel is reloaded (with 250 lbs. sand) ready for next morning.

* Bastien and Allen 30 Kw. electrode boiler, operated at 80 lb. p.s.i.

PLATE G

Zinc and copper deficiencies in tomato.

Left : **Zinc deficiency.** *Centre :* **Control.** *Right :* **Copper deficiency.**

[*To face page* 179

4.30 p.m. ... Leaching vat is filled with tap water and allowed to drain rapidly. Refilled with leaching solution of appropriately purified basal nutrient solution or calcium nitrate solution of 0·02 molar concentration and left to stand overnight, with small bung in exit of drainage tubulure. Vessel is adjusted with spirit level to vertical. Site is cleared.

When a batch of sand is to be treated for 3-hour periods with three separate changes of acid the following procedure is adopted and two leaching vats are required. At the end of the first day the sand that has been treated as described above is transferred to one vat and fresh sand loaded as described. This batch is then treated the following day and unloaded into the second vat. The first batch is then reloaded that evening and treated for 3 hours on the following morning until midday, when the acid is drained away and the sand unloaded into the vat whilst the steam pressure is kept at 1 lb. pressure. The second batch is then transferred from the other vat into the vessel and treated as described for the rest of the run. This procedure avoids leaving acid-saturated sand in the apparatus overnight, and for the same reason sand that is to be treated for two or more 6-hour runs must be unloaded at the end of the run each evening.

Slight modification of this routine is recommended when sand is required for molybdenum deficiency. The sand is treated for 3 whole-day runs, either with acid as described or first with acid, then with 5% sodium carbonate and finally with acid for the third run. The sand is leached with demineralised rain water after the first day's run, distilled water after the second and glass-distilled water at the end of the treatment. The final leaching is carried out in the glass pots and not in the vat. A small amount of sand is used first to "bed" down the watch glass and a layer of sand about 1-2 inches deep is added.

The beakers are half-filled with glass-distilled water and the remainder of the sand is then added, whilst an excess of water is maintained. This technique eliminates air pockets and uneven packing of the sand and ensures thorough leaching in the same manner as the elution of a properly packed chromatographic column, and the retention of traces of molybdenum is minimised.

Sand thus treated contained only 0·00002 p.p.m. or less of available molybdenum as determined by assay with *A. niger*. The course of molybdenum removal from sand during purification is illustrated in Table 18. It is not yet known whether use of alkali materially improves the quality of sand, but molybdenum deficiency may be more acute after an alkaline treatment has been included. A surface active material Perminal BX* is also included in some instances, as it may be expected that improved penetration of the acid or alkali into crevices in the sand grains is thereby achieved. Purified sand that has been treated for two days with acid has been used to produce acute copper and zinc deficiency in several crops.

In recent work the sand in the pots has been thoroughly leached by allowing a stream of tap water from a glass jet to flood through the sand in an upward direction until all suspended matter is removed. The sand is also free from all air pockets and is leached with purified calcium nitrate in the normal manner. Care is necessary not to displace the watch glass.

The removal of acid from purified sand presents certain difficulties as the sand is saturated with adsorbed hydrogen ions in addition to free acid. This adsorptive property was also observed by McHargue (1923 b) and a similar adsorptive capacity for other ions was noted by Arnon and Meagher (1947). Its importance lies in the fact that the exchangeable acid is only liberated when the sand comes in contact with nutrient solution and is sufficient to cause damage to roots of many plants. The method used to remove the acid is first to leach with water to remove free acid and then to leach the sand with nutrient solution (appropriately purified for the particular deficiency for which the sand is to be used). This leaching is carried out first in 20-gallon glazed leaching vats, as already described, or in the pots, using 1 L. of nutrient solution morning and evening until there is no change in pH

* Supplied by Messrs. I.C.I. Ltd., Hexagon House, Manchester.

on standing overnight ; Bromo-cresol green indicator is used for this purpose. Sand treated in this way does not give rise to injury due to adsorbed hydrogen ions, but it is advisable to flush the sand daily for two or three days after planting.

Effectiveness of Purification

Sand purified in the manner described is suitable for the study of deficiencies of iron, manganese, copper, zinc, boron and molybdenum in many crops when other necessary precautions are taken, and pronounced effects may be obtained. Some difficulty was experienced initially in the production of iron-deficiency and results were not always satisfactory when once-treated sand was used. Some plants, particularly rape, clover, sugar beet, flax and tomato showed marked recovery from initially severe symptoms. The distribution of the iron impurity partially occluded in crevices in the grains, already referred to in Part One, may afford some explanation of this recovery, as complete removal of iron from these regions may be difficult. Slow diffusion of iron subsequent to purification may continue, either as the result of traces of acid remaining in the crevices or from the action of nutrient solution. It is obvious that complete leaching of all the impurities brought into solution by the acid is the key to the preparation of a satisfactory batch of pure sand. The extension of the root system or more efficient contact between the root and the sand grains may also result in increased absorption of various micronutrients from imperfectly purified sand with consequent recovery.

WATER

Collection and Storage of Rain Water

The mineral composition of rain water has already been dealt with. The data presented show that although low the mineral content may still be significant and may vary with the site of the collecting surface and time of year. The nature of the collecting surface and storage tanks also affects the mineral content.

Rain water used in the sand cultures is collected from the glass roof of the main culture house, which presents an effective area of about 3,500 square feet. When the roof has been freshly painted or in any way interfered with, the rain water falling from it is allowed to go to waste until heavy rain has fallen, after which collection is resumed. The water runs into bitumen-painted gutters and thence into galvanised storage tanks also painted with bitumen. The gutters are cleaned out at intervals.

New tanks are weathered in the open air for a year before painting. At the end of this period the weathering process is hastened artificially by washing or spraying the galvanised surface with a 4% acetic acid solution. Particular attention is given to all rivets, seams and angles. The tanks are then washed out and dried before painting. At least three coats of paint are given annually for two years, after which the number is reduced to two. Failure to weather new tanks before painting may result in stripping of the paint film, particuarly along seams where accumulation of flux left after galvanising may occur. The accumulation of zinc in water stored in painted new tanks is much greater than in weathered tanks. The paint used is the same as that used for pots and other purposes.

The storage tanks are of about 500-gallon capacity each. Some are raised up from the ground sufficiently to allow a gravity feed through rubber hosepipe to parts of the greenhouse. Tanks are arranged in groups and are interconnected by hosepipe connections to " T "-piece outlets. These are located about 3 inches above the bottom of the tank to prevent the withdrawal of sediment that may collect on the bottom. This sediment carries down much of the iron and manganese in the water. Each tank has a threaded drainage hole in the bottom at the opposite end to the outlet and the tanks slope slightly towards the bung to allow for drainage and cleaning.

Small " service " tanks of 60-100 gallon capacity are placed at convenient points in the greenhouse for storage of water needed for daily use. Separate tanks are kept for water used for different experiments.

Consumption in Relation to Storage Capacity

For vegetables a consumption of 12-15 gallons per 10-inch pot for a period of 4-5 months appears to represent the average for a wide variety of treatments. Thus 1,000 cultures consume at least 12-15,000 gallons of water during an average growing season. To meet this demand a storage capacity of 9,000 gallons filled at the beginning of the season is provided. Reliance is placed on the rainfall during June, July and August to supply the remainder.

A rainfall of 1 inch on an area of 1,000 square feet represents about 520 gallons of water. Of this, however, considerable amounts are lost by evaporation, especially during small showers in warm weather. Increase in the number of cultures means a disproportionate increase in storage capacity, if the collecting area remains the same.

Suitability and Purification of Rain Water

Rain water collected and stored as described above is not toxic and may be used without further purification for many purposes. These include experiments designed to *produce* symptoms of deficiencies of nitrogen, phosphorus, calcium, magnesium and potassium and toxicities of manganese and aluminium in all crop plants so far studied. Greater severity of calcium deficiency may be obtained by using purified rain water. For precise experiments where quantitative results with levels of calcium or potassium below 1-2 p.p.m. are required, or where studies of micronutrient problems are undertaken, the rain water must be further purified and such treatment also effectively avoids any chance of zinc toxicity or other injurious effects. For this purpose a procedure using the Permutit synthetic resin ion-exchange method of demineralisation has been developed and is described below. Rain water treated in this manner is used for study of copper, zinc, molybdenum, iron, manganese and boron deficiencies, for which it has proved entirely satisfactory, and no toxic effects have been observed or suspected.

The equipment used at present comprises five portable ion-exchange units. The first, third and fifth are Zeocarb cation-exchanging units, while the second and fourth are De Acidite anion-exchanging units. The flow of water is upwards through each and is led through acid-extracted rubber and glass tubing controlled by screw pinch clips. " T " pieces are arranged so that the tubes provided to admit the regenerating solutions and carry off the wastes can be interposed at suitable points and this arrangement permits of great flexibility in use, as any unit can be isolated and by-passed if required, whilst the charging of the siphons from the jars of regenerating solutions is done automatically. The layout is shown diagrammatically in Figure 8 with the Biodeminrolit unit for copper, zinc and molybdenum removal.

The supply to the Permutit water purification equipment is provided either from the elevated storage tanks, which give a " head " of only 2-6 feet of water, or from an overhead tank of 50 gallons' capacity. This can be filled either by tap water or by rain water raised through a small stainless steel rotary pump driven by an electric motor and controlled with a float switch mechanism so that the tank is refilled when sufficient water has run through the Permutit units.

The units are arranged in a row on a stand 3 feet from the ground. If required, the incoming water is metered in a rotameter to allow control of the rate of flow. The effluent from the last (cation-exchanging) unit is led through glass tubing to a " chain " of several 10-gallon glass carboys which serve as a buffer storage. These carboys have been used for the storage of concentrated hydrochloric acid prior to their use for this purpose. Pyrex or hysil glass carboys would be an added refinement. The carboys are interconnected by wide bore glass siphon tubes. When water is withdrawn the arrangement of the carboys is such that each carboy in the chain is fed by 2 carboys siphoning into it. This arrangement has been devised to prevent the siphon " breaking " due to the withdrawal of water for use more rapidly than the siphons can supply the last carboy. The last carboy is inverted so that water is withdrawn for use without delay by direct flow. Each carboy is fitted with a watertight rubber bung ; this and all rubber connections are previously boiled in 1 : 3 hydrochloric acid and rinsed in distilled water, to remove metallic impurities.

A glass airline leads from the bung to a height slightly above the maximum level of water in the elevated feed tank and primary storage tanks. When the carboys are filled the water level ascends the airlines until it reaches the level in the main storage tanks, when the flow ceases. The airlines can also serve as a gauge for the level in the main storage tanks. As soon as water is withdrawn the water level in the airlines drops and inflow recommences. Thus it is possible to withdraw rapidly 3-500 L. of purified water and the supply is replaced during the remainder of the day and night by the relatively slow inflow permitted by the size of the units, until the carboys are refilled. The rate of inflow depends on the available head, but should not exceed 20 gallons per hour for rain water (or 8 gallons per hour for tap water). The buffer storage is thus not stagnant and may be designed to suit the requirements of the experiments in progress.

The rubber outlet of the last carboy is controlled by a specially designed home-made clip. This clip is made from hard wood and metal strips and rods (preferably of stainless steel). The clip was especially designed to have a wide " jaw," automatic spring closing, and an accurate and tight grip on the tubing, and to avoid the contact of any metal with the rubber. Wood, bakelite or wide-bore glass stopcocks would probably be equally satisfactory.

Operation of Permutit equipment. The working instructions given below are based on experience and cover the operation and regeneration of the equipment. The details refer to the use of an installation with three units. The additional procedures involved when five units are present are also indicated. Regeneration is required in order that the resins may be freed of adsorbed mineral ions ready for further demineralisation. The quantity of water that can be treated before this procedure is necessary depends, as stated earlier, on its mineral content. Thus the capacity of the second pair of units is greater by about 8 times than that of the first pair and that of the last unit is correspondingly greater still. About seventy-five gallons of Bristol tap water (a hard water) may be treated before the first two units require regeneration, whilst nearly one thousand gallons of rain water can be purified safely before regeneration is necessary. In practice the second pair of units are only regenerated about once in five times for the first pair and the final unit is only regenerated at the end of every season. The layout is shown in Figure 8.

The use of soft water causes prolonged persistence of sodium ions in the effluent from the second and fourth units after regeneration and a process of alternate rinsing and standing has been adopted to reduce the amount of water required to complete the rinsing of these units after regeneration.

" Working Instructions " for Operation of Demineralisation Apparatus, based upon an Installation of Three Units [Figure 8]

A. Rain Water

1. Close (1)* and (10) and see that tap water control valve, if provided, is closed.

2. Arrange regenerating reservoirs (three in all) as shown and charge siphon lines as follows : open rain water control valve and (1), open and close (2), (5) and (8) in turn to fill siphon lines.

3. Close (1), (4) and (7).

4. Open (5) and (6) and allow contents of reservoir to siphon through respective units ; close (5) and (6) as soon as reservoir is empty.

5. Open (2) and (3) and proceed as for 4 above.

6. Open (1) and (2) and flush siphon line. Close (2).

7. Open (3) and rinse No. 1 unit with approximately 5 gallons at 6-8 gallons per hour (until pH of effluent is constant, probably between pH 3 and 4). Close (3).

8. Open (4) and (5) to flush siphon line. Close (5).

---

* Bracketed numerals refer to number of valves or clips in flow sheet.

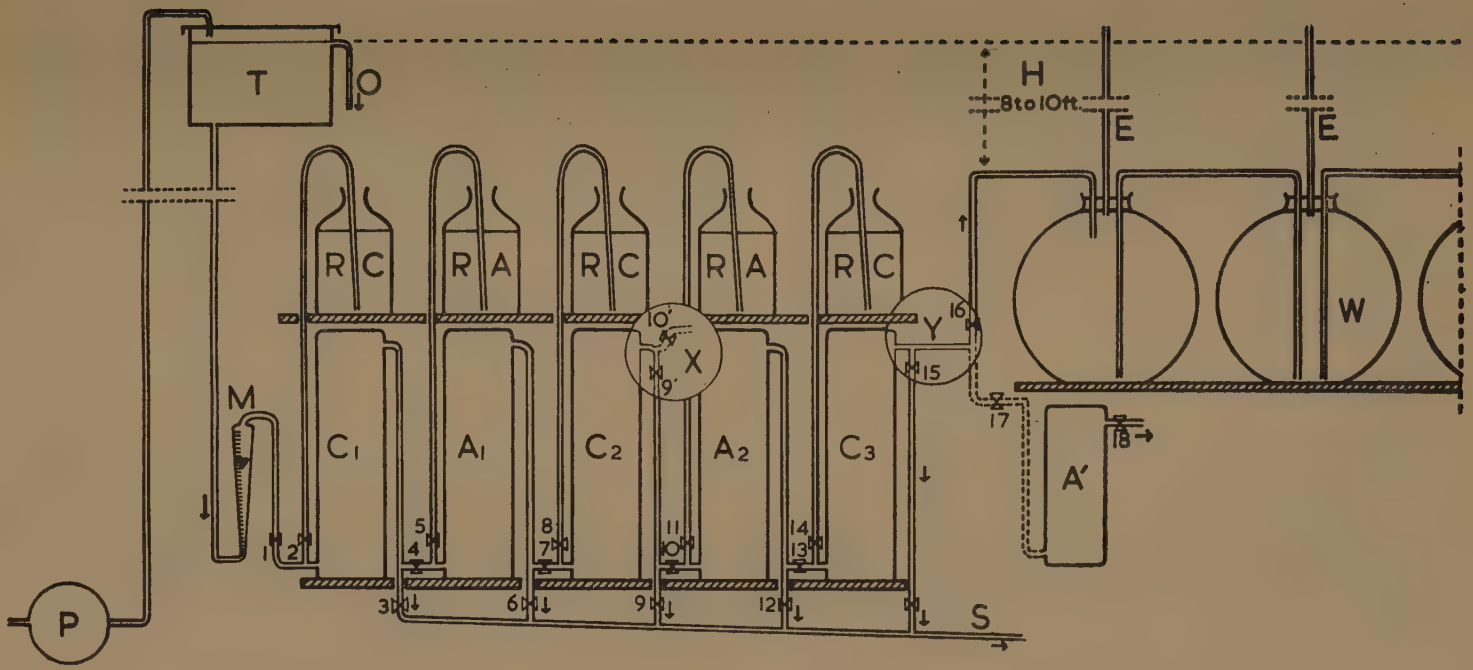


FIGURE 8

Diagrammatic lay-out and flow sheet for use of ion-exchange water purification equipment for 5 units

A_1 A_2 = Anion removing units
A^1 = Special anion or mixed bed resin unit for weakly dissociated ions, e.g. molybdate.
C_1 C_2 C_3 = Cation removing units.
RA = Reservoirs for regenerating solution, N.NaOH.
RC = Reservoirs for regenerating solution, 2N. HCl.
P = Pump.
T = Tank.
O = Overflow.
M = Rotameter flow-meter 1-10 g.p.h.
S = To waste.
H = Head, 8 to 10 ft.
E = Air outlet tubes.
W = Purified water storage carboys with connecting siphons.
X = 3-unit assembly.
Y = 5-unit assembly.

Numerals refer to stopcocks in text, pp. 189.

9. Open (6) and rinse with 20 gallons at 6 gallons per hour and at the same time open (8) and (9), and allow reservoirs to siphon through No. 3 unit, then close (8) and (9).

10. When 20 gallons have passed through No. 2 unit, close (6) and open (7) and (8) to flush siphon line, and close (8).

11. Open (9) and rinse with 5 gallons. Close (7) and (8) and leave to stand overnight.

12. The following morning open (6) and rinse with 5 gallons at 6 gallons per hour. Close (6) and allow to stand about 5 hours, and then repeat the operation. Close (6).

13. Open (7) and (9) and rinse at 3 gallons per hour for about 3 hours.

14. Close (9) and open (10) and collect at rate of 5-15 gallons per hour (for rain water).

Purification and collection are now automatic, but it is advisable to rinse No. 2 unit only once or twice by repeating operation No. 12 during the first one or two days after regeneration, to complete the removal of sodium, which tends to persist when soft water is used.

B. Tap Water

1. Close (1) and (10) and see that rain water control valve is closed. Fill overhead tank with 30 gallons of tap water.

2. Proceed as for rain water up to operation 8 inclusive.

9 and 10. As for rain water, but using 15 gallons only.

11. As for rain water.

12. As for rain water, using 2 gallons and leave to stand for shorter interval of 2 hours.

13. As for rain water for 1 hour.

Collection

14. Fill overhead tank with 70 gallons.* Open (10) and collect at 6-8 gallons per hour.

15. After 60 gallons has been collected close (1) and (10). Regenerate No. 1 and No. 2 units only and proceed as above, but *omit any rinsing through No. 3 unit.*

No. 3 unit should be regenerated once for each five runs through Nos. 1 and 2 units.

Additional instructions for use of five units. The fourth and fifth units are an anion and a cation remover respectively and are connected in the same manner as the preceding three with the exit at (10) connected to the base of No. 4 unit. No. 4 unit is regenerated on each occasion that this operation is carried out for No. 3 unit. No. 5 unit is only regenerated once for twenty regenerations of Nos. 1 and 2 units (i.e. once for four regenerations of Nos. 3 and 4 units) or at the end of the season. Nos. 3 and 5 units are regenerated in the same way as Nos. 1 and 2 units (given above) and the points with regard to the leaching of No. 2 unit apply equally to the No. 4 unit. No. 5 unit is regenerated as described originally for No. 3 unit. Collection is carried out in exactly the same manner as before.

Table 22

Composition of Permanent Artificial Standards for Manganese Test (Final Volume 13 ml. each)

Indicator	Volume ml. B.D.H. standard solutions										
Bromo-thymol blue	·035	·060	·09	·125	·125	·150	·20	·25	·30	·40	·50
Thymol blue	—	—	—	—	·025	·050	·10	·10	·15	·15	·20
Bromo-phynol blue	—	—	—	—	—	—	—	·05	·10	·15	·20
H_2O (1% NH_4OH)	13	13	13	13	13	13	15	18	18	18	20
Mn = in p.p.m.	2–3	5	10	15	20	30	40	50	60	70	80

* This figure is appropriate for Bristol tap water which may contain up to 120 p.p.m. of calcium and magnesium. Softer water supplied may be treated in greater amounts before regeneration is needed.

The quality of the demineralised water does not vary appreciably until break-through is imminent and the question of a suitable test of the need for regeneration has already been discussed. Reliance is placed on the data obtained from trial runs when tap water is used to remain within the calculated safe limits. For demineralised rain water a check, however, can be made with the tetrabase test for manganese carried out as described below. Recent tests with the dithizone reaction for heavy metals have also shown that in spite of the use of galvanised tanks the effluent may often satisfy the exacting requirements of this test carried out under the sensitive conditions given below.

Tetrabase test for manganese in unconcentrated samples of water ; range 0·001-0·1 p.p.m. Mn :—

REAGENTS

Tetramethyldiaminodiphenylmethane (" tetra base ") recrystallised from aqueous methyl alcohol : 0·1% solution in acetone (redistilled). Stored not more than one week in dark. Potassium periodate : Saturated solution of recrystallised salt, in glass distilled water ; stored in pyrex or hysil glass.

Sodium acetate-acetic acid buffer (Morgan's reagent pH 4·6-4·8) prepared from sodium acetate (10% solution) purified by alkaline phosphate absorption as for nutrient reagents (1.c.) and 30 ml. of redistilled acetic acid per litre, added after purification and stored in pyrex or hysil glass.

All glassware cleaned in 1 : 1 hydrochloric acid and glass distilled water. Tests are carried out in 4″ × 1″ flat-bottomed tubes and compared with synthetic standards prepared as shown in Table 22 :—

Procedure. 10 ml. of test solution is followed in order by 2 ml. of Morgan's reagent, 2 ml. of periodate solution and 1 ml. of tetra base solution. The blue colour that develops is matched against the standards when the maximum *blue* colour development is obtained or until a trace of red-purple tint (for higher concentrations) is just detected. The preparation of the standards is based on the use of the test at normal air temperatures in daylight. Blank determinations should be made and should not exceed 0·001 p.p.m. Mn. The standards are stable in the dark for about three months if stored in an atmosphere free of acid fumes. Values found rarely exceed 0·001-0·002 p.p.m.

Dithizone test for heavy metals :—

REAGENTS

1 L. W/W 0·05% Diphenylthiocarbazone (Dithizone) dissolved in redistilled carbon tetrachloride and purified as follows.

The dithizone is dissolved in carbon tetrachloride and filtered. The solution is placed in a clean 2-L. pyrex separating funnel with 1-L. glass-distilled water and a few drops of pure ammonia solution to produce a concentration of approximately ·02 N. The funnel is shaken vigorously until most of the dithizone has passed into the aqueous phase. Most of the heavy metal impurities are retained in the carbon tetrachloride, which is run off. More solvent is added and extraction and separation are repeated until the carbon tetrachloride layer remains pale green. 500 ml. of redistilled carbon tetrachloride are added with a few drops of redistilled hydrochloric acid, sufficient to produce an acid reaction equivalent to ·02 N. The funnel is shaken vigorously to extract the purified dithizone into the solvent which is run off into a pyrex container and made up to 1 L. with redistilled carbon tetrachloride and the solution is stored in a refrigerator.

Procedure. Calibrate a 125-ml. pyrex or hysil conical separating funnel at the 5-, 10- and 100-ml. levels, clean rigorously in hot 1 : 1 hydrochloric acid and glass-distilled water, shake with a solution of dithizone in carbon tetrachloride, and reject this fraction. Rinse out with a few ml. of carbon tetrachloride.

Introduce 10 ml. carbon tetrachloride and 0·1 ml. of dithizone solution, shake and run out to 5 ml. Add water to be tested to 100 ml. and shake vigorously.

A green colour indicates that contamination is less than 0·001 p.p.m. total heavy metals.

A pink colour indicates heavy contamination, greater than 0·02 p.p.m.

Blue-green and purple indicate intermediate concentrations. pH value should be 5-6.

Distilled Water

Metal Stills

The distilled water used for the cultures at Long Ashton is obtained either from tinned copper stills or from continuous-operating pyrex glass stills. The tinned copper stills are gas or electrically heated and deliver 4-4·5 litres per hour maximum. They have tinned copper, solid silver or pyrex glass condensers. The distillate is stored in 50-L. carboys fitted with glass siphons to withdraw water for use and covered to reduce contamination by dust. The water is softened prior to entering the stills, as with hard water the condenser and boiling pan readily becomes covered with " rock " which produces a fall in efficiency and eventually causes insufficient cooling of the distillate which becomes very hot. The iron, manganese and boron contents of water from the tinned copper stills with tin condensers are usually 0·004 p.p.m. or less. The zinc and copper content may be 10-20 times as great.

Three stills with solid silver condensing surfaces and steam guides designed by Brenchley and used at Rothamsted have also been installed. Before use rigorous cleaning of the silver surfaces was found to be needed. This was done with best quality solid silver cleaning powder and distilled water until all marks were removed. The guide and angular gutters were also similarly cleaned with material wound over a slender holder and examined with a light and mirror. These stills are electrically operated with self-protecting elements. It was hoped that this method of heating would considerably reduce the excessive and highly contaminating products of corrosion that result from the continuous use of gas burners. Bright blue corrosion products are still produced, however, where the warm condenser water enters the boiling pan. The distillate contains about 0·00006 p.p.m. molybdenum and appreciable concentrations of heavy metals that react with dithizone. Water obtained from electrically-heated metal stills with pyrex glass condensers is not suitable for work on copper or zinc deficiencies, as shown in Plate F.

Glass Stills

The pyrex glass stills* shown in Figure 3 and photograph are designed for continuous operation. The gas-operated stills have an output of 1·3-1·6 L. per hour ; the output of the electrically-heated ones is about 0·65 litre per hour with a 650-W. bowl fire element ; this can be rewound for 1 Kw. The constant level feed is adapted from the Mariotte's bottle design introduced by Piper and Oertel and described in Part One. A 50-litre carboy (preferably of pyrex glass if used to supply water for a second distillation from glass) and a buffer aspirator of 2-L. capacity replace the large aspirator used by Piper and Oertel. The carboy acts as a Mariotte's bottle, but the water leaves via a siphon instead of a basal tubulure. The whole success of the apparatus depends on the airtight fit of the bung and all the rubber-glass connections *above* the siphon level. The small buffer aspirator is necessary to compensate the back pressure caused by rapid boiling which would otherwise cause the siphon supply to " break " and the boiling flask could then run dry. Each small aspirator feeds two stills. The position of the base of the air line controls the level of water in the still and should be adjusted to give a level approximately 1·5 cm. *above* that required to be maintained when the still is in use. This is due to the effect of the steam pressure in the flask which exerts a back pressure against the inflow. Failure of the condenser cooling water has a similar effect and may endanger the boiling flask.

The double splash tap is thought to be effective in reducing contamination from spray and creep and does not impair the rate of distillation owing to the wide bore and the vestibule

* These stills were made by Mr. J. H. Burrow, B.Sc., of the University of Bristol Physics Dept., and his kindness is gratefully acknowledged.

in the condenser. All ground glass joints are standard and allow of interchange of parts. The distillate is conducted to pyrex glass carboys plugged with cotton wool to exclude dust.

The Mariotte's bottle reservoir is filled with the aid of a suction pump direct from a carboy supplied by the " silver " stills. This procedure can be carried out while the stills are running by closing the air line, siphon clips and stopcocks and opening those controlling the evacuation and filling lines. When full the evacuation and filling lines are first closed, the pressure release is opened and the pump turned off. (A trap is essential between the pump and the evacuation line.) The air line (to the bottom of the carboy) is then opened. The air release is closed and the siphons to the stills re-opened.

A check is necessary to see that during evacuation the siphons have not been " broken " as this would cause the stills to run dry. Should this occur they are refilled by blowing air into the carboy through the air release which is opened for the purpose. The open ends of the siphon tubes inside the carboy should be some distance away from the base of the air line, as otherwise air bubbles lodge in these tubes and cause failure of the water supply through the siphons. The bottom of the air inlet is turned aside at a slight angle. When not in use it is customary to close the clips controlling the siphons in case of slight leakage which would permit water to continue to enter the stills overnight and fill them above the still heads. As a precaution against contamination of a batch of distilled water by this means the highest point of the still head should be just above the level of water in the carboy when full. The siphons are checked first thing in the morning to ensure that no air bubbles have collected as air enters slowly where the rubber becomes perished.

Water obtained from the glass stills usually passes the dithizone test and is satisfactory for the study of copper, zinc and molybdenum deficiencies. The molybdenum content is approximately 0·000003-5 p.p.m. The water is stored in 60-L. pyrex glass carboys in strong baskets. Water is delivered through a wide-bore glass siphon, closed either by a clip, as described earlier, or by a 12-mm. bore stopcock.

Tap Water

Tap water is used without further treatment in a few experiments where low (or differential) levels of nutrient elements are not involved. No evidence of toxicity has been observed.

Tap water can be purified by the same ion-exchange equipment already described, should rain water supplies fail in an exceptionally dry spell. Owing to the hardness of tap water only 75 gallons can be treated by the first pair of units before regeneration and the procedure is thus rather laborious. A small overhead tank with a float gauge provides a fairly constant head. The limited supply also avoids accidental break-through if the tap water is not turned off after 75 gallons and safeguards water already collected. The purified tap water is stored in 200-gallon tanks. The details of operation are similar to those given for rain water and are included in the schedule of working instructions. Tap water purified by the ion-exchange method contains less than 0·5 p.p.m. total calcium and magnesium. Removal of iron is less efficient than from rain water as the iron is present as ferrous bicarbonate which is not readily removed by ion exchange. As removal of weak acids like boric acid is of low efficiency, the boron content is only reduced by about 60% for each anion unit. Tap water as obtained at Long Ashton after purification has proved satisfactory for the study of all macronutrient deficiencies and also those of iron, manganese and boron in many plants. Demineralisation may also be expected to remove traces of injurious chlorination products.

NUTRIENT REAGENTS

Composition and Preparation of Nutrient Solutions

The standard complete nutrient solution used in this work was derived from nutrient solutions used at Long Ashton to grow fruit trees. The complete nutrient produces vigorous growth in a wide range of crops and no attempt has been made to develop numerous " optimum " complete nutrient solutions to suit the various needs of different crops. Two main types of nutrient solution differing only in the Ca/K ratio are used. A corresponding difference is

maintained in the deficiency solutions derived from them, i.e. calcium-deficient nutrient solutions, for example, vary with respect to the Na/K ratio, as sodium is used to replace calcium. The majority of crops now receive a nutrient solution with a Ca/K ratio of 2 : 1 equivalents per litre. The composition of the standard complete nutrient solution is given later. The nutrient solutions are prepared, as described later, by mixing the appropriate volumes of concentrated stock solutions. The complete nutrient solution does not precipitate at ten times the required concentration, even on long standing, provided it is made up by dilution into a large volume of water and is stored in the dark or in brown glass bottles.

Although nutrient elements and their interrelationships are often discussed in terms of their concentrations as parts per million, the concentrations of elements supplied are adjusted to correspond to simple multiples or fractions of milligram equivalent weights per litre for two reasons. Calculation of concentrations of stock solutions and the volumes required for the preparation of any given nutrient solution is thus simplified and is more fundamental, as explained in Part One. The standard unit of concentration becomes the milligram equivalent weight per litre referred to as m.eq./L. except for some elements where the millimol is more suitable at present. The reference to concentration in terms of parts per million is also retained for convenience for comparative purposes.

Iron was originally supplied at 15 p.p.m. as ferric chloride. This, however, resulted in a low pH of about 3·6 due to hydrolysis, or to reaction with the sodium dihydrogen phosphate with the formation of hydrochloric acid. Although excellent growth was obtained with such solutions, the ferric chloride was replaced by ferric citrate and iron was supplied at 5·6 p.p.m., i.e. 0·3 m.eq./L. This eliminated the drop in pH and prevented precipitation of phosphate. The nutrient solutions which contain citrate have a pH between 5 and 6 and this may increase to 7-8 after application. No symptoms of iron-deficiency have occurred in these sand cultures unless iron has been omitted.

The growth of fungi in the stock ferric citrate is rare when stored in darkened glass bottles and is prevented completely by suspending from the stopper of the stock bottle a bag of bolted silk containing solid ethyl mercuric chloride. For highly purified solutions no such precautions are taken. Micronutrients are incorporated in the nutrient solution when prepared and are thus included at each application. Copper, zinc and molybdenum are always included, since it is possible that one of these elements may accidentally become limiting, especially if refined materials are used. Aluminium, gallium, cobalt and nickel are also included in highly refined experiments on account of sundry references to their essential nature for plants. The stock solutions of nutrient salts used for many experiments are prepared from " recrystallised " grade reagents for the major components and from those of analytical reagent quality for micronutrient supplements. Purified nutrients prepared from analytical reagent grade compounds as described below are used for work on micronutrient deficiencies.

In the large-scale experiments the concentrated nutrient solutions are diluted to 10 litres in 10-quart glazed stone jars and the contents further diluted 1 : 9 before application. Precipitation is avoided (as already stated) by dilution of the respective stock solutions into water already in the stone jars. For convenience in handling, the stone jars are used to fill smaller bottles from which the solution is diluted immediately before use. One batch of concentrate is used to make 100 litres of nutrient solution ready for application. In the smaller-scale experiments one-fifth of the volume of stock solutions used for rain water types is diluted to two litres in pyrex glass bottles partly filled with water and is sufficient for twenty litres when diluted again before application. Mixing the concentrated stock solutions together without dilution produces a crystalline precipitate of calcium sulphate which redissolves only slowly after dilution.

The stock solutions of separate nutrient reagents are made up as concentrated " standard " solutions shown in Table 23 so that a convenient, stated volume (usually 50 or 100 mls.) contains the requisite weight of the reagent to produce a solution of (usually) 1-4 m.eq./L. with respect to the element concerned *after dilution to* 100 *litres*. In early work the volumes of stock solutions were measured out in graduated cylinders, but in 1946 several large, automatic burettes were installed to replace the cylinders. The burettes are connected to

reservoirs of concentrated stock solutions. The reservoirs are of 2-5 L. capacity and are of pyrex glass for the purified stock solutions. The burettes are filled with the aid of compressed air applied to the reservoirs from a small compressor. They are graded in diameter and conveniently calibrated for the range of volumes of each stock solution normally required in the different types of nutrients. Each is plugged at the top with cotton wool. Burettes used for micronutrient-deficiency solutions have glass stopcocks, while those used for less highly purified solutions are controlled by pinch clips and rubber tubing.

The use of the burettes (the jets of which are not allowed to come in contact with the bottles containing the mixed concentrates) reduces the possibilities of contamination, improves the accuracy of nutrient composition and effects considerable saving in time in the preparation of a great variety of nutrient solutions.

Calcium, magnesium and potassium when omitted are normally substituted by the equivalent amount of sodium, and when sodium is omitted it may be substituted by potassium or calcium according to the experiment. Nitrates and phosphates are substituted by sulphates. Thus by reference to Table 23 the equivalent volume of sodium nitrate to replace potassium in a solution containing 3·33 m.eq./L. is seen to be 87·5 ml. and for replacement of calcium in a solution containing 6·67 m.eq./L. the volume is 165 ml. Similarly, a solution lacking phosphate is prepared with an equivalent amount of sodium sulphate, i.e. 133 ml. supply 1·33 m.eq./L. Na, whilst the volume required to replace the standard amount of magnesium sulphate is 300 ml., i.e. 3 m.eq./L. Na.

When calcium sulphate is required, it is added as a standard suspension measured by volume from a cylinder.

Nutrient salts are not normally examined for the presence of impurities of macronutrients such as calcium, potassium and magnesium ; the calcium-deficient nutrient solution, however, may contain 0·25 p.p.m. of calcium derived mainly from the technical or recrystallised grade salts. This concentration does not prevent the development of severe calcium-deficiency symptoms in most crops examined, but may cause slight delay in their appearance. The composition of the standard complete nutrient solutions is given here :—

pH 5-6

Salt	Wt. in g. (for 100 litres of diluted nutrient)
KNO_3	20·2 or 33·6 or 50·5
$Ca(NO_3)_2$ (Anhydrous)	65·6 or 54·7 or 82·0
$NaH_2PO_4 2H_2O$	20·8
$MgSO_4\ 7H_2O$	36·9
Ferric Citrate	2·45
$MnSO_4\ 4H_2O$	0·223
$CuSO_4\ 5H_2O$	0·024
$ZnSO_4\ 7H_2O$	0·029
H_3BO_3	0·186
$(NH_4)_6Mo_7O_{24}4H_2O$	0·0035
$(Al)_2SO_3\ 12H_2O$	0·0186
$Ga(NO_3)_3$* 1% solution of Ga. ...	0·05 ml.
$CoSO_4\ 7H_2O$	0·0028
$NiSO_4\ 7H_2O$	0·0028

This gives the following concentrations as milligram-equivalents per litre (m.eq./L.) :—

NO_3' 10 (2 + 8 or 3·33 + 6·67) or 15 (10 + 5) ; PO_4''' 4 ; SO_4'' 3 (neglecting that due to micronutrient contributions) ; Ca^{++} 8 or 6·67 or 10 ; Mg^{++} 3 ; K^+ 2 or 3·33 or 5 ; Na^+ 1·33 ; H^+ 2·67 ; Fe^{+++} 0·33 ; Mn^{++} 0·02 ; Cu^{++} 0·002 ; Zn^{++} 0·002 ; B 0·033 (milli mols.) ; Mo 0·0002 (milli mols.). Al^{+++} 0·001 ; Ga^{+++} 0·0002 ; CO^{++} 0·0002 ; Ni^{++} 0·0002 ;

* The gallium nitrate solution is obtained from Messrs. Johnson Matthy Ltd., and is one of the standard " Specpure " grade compounds.

or as parts per million (approximately) :—

N 140-210 ; P 41·3 ; S 48 ; Ca 160 or 133·4 or 200 ; Mg 37 ; K 78 or 130 or 195 ; Na 30·7 ; Fe 5·6 ; Mn 0·55 ; Cu 0·064 ; Zn 0·065 ; B 0·37 ; Mo 0·019. Al 0·009. Ga 0·005. Co 0·006. Ni 0·006.

These concentrations exclude impurities introduced by water, etc.

It will be seen that the nutrient solutions used at Long Ashton are based on nitrate as a source of nitrogen. Ammonium compounds are not normally included, partly in order to avoid the possibility of toxic effects, and also because of the complication produced in relation to experiments on the availability of iron, where it may be necessary to study the effects of other types of nitrogen supply. The method of using ammonium compounds to control pH variations in the nutrient solutions is thus precluded. It is felt that, where pH control is required, constant flowing cultures are the proper approach to the problem.

In some experiments solid calcium carbonate may be used as a source of or as a supplement to calcium supply. Finely powdered, pure, precipitated grade calcium carbonate is mixed intimately with the sand spread out in a shallow layer. The usual rates given are from 10-100 g. per ten-inch pot. Magnetite when given is incorporated at 0·1% concentration by weight.

TABLE 23

Concentration of Standard Stock Solutions

	Concentration %	G. weight dissolved in 2 litres of solution	Milligram equivalents per litre in 100 litres per 100 mls. of stock
KNO_3	20·2	404	2
$NaNO_3$	34·0	680	4
$Ca(NO_3)_2$ Anhyd.	32·8	656	4
$Mg(NO_3)_2$	22·25	445	3
NH_4NO_3	16·0	320	2 (N as N = 4)
$(NH_4)_2SO_4$	26·4	528	4
Urea	30·0	600	10
K_2SO_4	8·7	176	1
Na_2SO_4, $10H_2O$	16·1	321	1
$CaSO_4$, $2H_2O$	— 34·4% W/V *suspension*	688	4
$MgSO_4$, $7H_2O$	18·4	368	1·5
KCl	14·9	298	2
NaCl	11·7	234	2
$CaCl_2$ Anhyd.	11·1	222	2
NaH_2PO_4, $2H_2O$	20·8	416	4 as PO_4'''
NaH_2PO_4, $12H_2O$	11·95	239	1 ,,
$Ca_2(H_2PO_4)_2$	15·45	309	4 ,,
$(NH_4)_3PO_4$, $3H_2O$	13·55	271	2 ,,
Ferric Citrate	4·9	98	0·6 as Fe^{+++}
$MnSO_4$, $4H_2O$ (Toxicity expts.)	22·3	446	2
$Al_2(SO_4)_3$, $18H_2O$ (Toxicity expts.) ...	22·2	444	2
(Micronutrients)			
$MnSO_4$, $4H_2O$	2·23	44·6	·02 in 10 *mls.*
$CuSO_4$, $5H_2O$	0·25	5·0	·002 ,,
$ZnSO_4$, $7H_2O$	0·29	5·8	·002 ,,
H_3BO_3	1·86	37·2	·03 milli mols. in 10 *mls.*
$(NH_4)_6Mo_7O_{24}$, $4H_2O$	0·035	0·7	·0002 milli mols. in 10 *mls.* (i.e. ·0012 m.eq./L. of hexavalent Mo)

It has been found an advantage to increase the total nitrate nitrogen level by 2-5 m.eq./L. for all crops and by 10-15 m.eq./L. for some *Brassica* crops. This is achieved by the supply of additional calcium and potassium nitrates in proportional amounts, as shown in the third column of the nutrient solutions above. Although no trials have been made it is likely that the copper concentration could be reduced to 0·001 m.eq./L. (i.e. 0·032 p.p.m.). There is reason to believe that the molybdenum level could be raised to 0·0005 milli mol/L. (i.e. 0·048 p.p.m.) with advantage, and that some crops will benefit by even higher concentrations (Hewitt and Jones, unpublished; Agarwala (1951)).

The Preparation of Nutrient Solutions for Micronutrient Deficiency Work

Analytical reagent or " Batch Analysis " grade salts are used where possible for the preparation of nutrient solutions for micronutrient deficiencies.

Purification of Stock Solutions from Iron, Manganese, Copper and Zinc

The stock solutions of appropriate nutrient salts are prepared in pyrex or hysil glass flasks to give the concentrations shown in Table 23 when made to volume, but slightly less water is used. To these solutions are added the following supplements in the volumes indicated per litre :—10 ml. 2 Molar (32·8%) calcium nitrate except to calcium salts. 10 ml. of Molar dipotassium hydrogen phosphate (17·6%) (or the sodium salt if all potassium must be omitted). 10 ml. of Molar (8·4%) sodium bicarbonate, and 20 g. of analytical reagent grade calcium carbonate. The solutions are swirled vigorously when the phosphate is added and again after the addition of the carbonate. The flasks are plugged with cotton wool or capped with a small beaker over the neck and autoclaved at 20 lb. pressure for 60 minutes. They are filtered, after standing overnight, through a large Buchner funnel kept exclusively for this purpose and cleaned with 1 : 1 hydrochloric acid (as also specified for all glassware). Two layers of Whatman No. 42 papers are used. The Buchner funnel is held in an adaptor designed to introduce the solution directly into the stock bottle and to avoid back air locks during evacuation. A glazed porcelain filter known as the " Minifilter " type B. 18·5 cm. is also used.* The filtrate is examined critically and should be crystal clear ; if this is not so the filtration is repeated. Previous wetting and proper bedding of the filter paper with a glass rod whilst suction is applied and choice of Buchner funnel with a flat base help materially in this respect. Once a layer of calcium phosphate and carbonate sludge has settled on the funnel, filtration is usually quite clear and a filter thus prepared can be re-used with advantage for a second filtration. The flask should be tilted gently to decant the supernatant liquid with minimum disturbance of the contents. A glass rod is used to steady the filter paper while the solution is poured on to it. The solutions are made up to volume in the calibrated 2-L. pyrex glass reagent bottles.

Removal of Copper and Zinc

When it is necessary to remove copper and zinc, further purification of the autoclaved solutions is effected by extraction with 0·05% dithizone solution in carbon tetrachloride purified as described previously (Dithizone test for heavy metals in water). Extractions are carried out at pH 6 in a 4-L. pyrex glass separating funnel until no trace of pink or blue-green colour appears in the carbon tetrachloride layer when a small portion is used after being diluted to approximately 0·005% concentration. The extracted solutions are then left to stand in the funnel for a few hours with an occasional light shake or swirl to complete the settling of the residual carbon tetrachloride. This is then run off before the solution is bottled and made up to volume. The carbon tetrachloride is removed by volatilisation on standing.

Purification of Stock Solutions from Boron

The removal of boron is most satisfactorily accomplished by recrystallisation. This procedure is suitable for such compounds as potassium nitrate, magnesium sulphate, sodium phosphate and other salts that crystallise easily. It is less convenient for dealing with calcium nitrate and this salt is obtained pure in a different manner as described later.

---

* This is manufactured by Messrs. Hathernware Ltd., Loughborough, Leicester.

The recrystallisation procedure is as follows :—1-2 kilo of the salt to be purified are dissolved in distilled water at a temperature of approximately 80° C. to produce an almost saturated solution. .The solid salt is added in portions with stirring in until solution is complete. The solution is heated to about 90° C. and filtered through a Whatman No. 41 paper on a Buchner funnel into a clean flask. The contents are then transferred to a clean 2- or 3-L. beaker. Crystallisation is allowed to proceed with stirring until the temperature has fallen to about 40° C. An equal volume of pure alcohol is added and the mass is kept stirred *until quite cool.* This is essential to prevent the formation of a solid rocklike mass of crystals which cannot be separated from the glass. The crystals are filtered on a large Buchner funnel or Minifilter. The mother liquor is cooled in a refrigerator to 0° C. and the remaining crystals are separated by decantation and added to the main crop if desired. The crystals are dried and pressed on a vacuum filter and redissolved in distilled water. The recrystallisation is repeated as described. The crystals are washed with purified alcohol and dried in a *low temperature oven* (not above 40° C.).

Magnesium sulphate and sodium phosphate may form two immiscible phases after addition of the alcohol to the warm saturated solution. This is overcome by vigorous stirring which eventually results in rapid crystallisation and the production of a single phase.

Two recrystallisations are sufficient for potassium nitrate and sodium phosphate ; three are used for magnesium sulphate. The procedure gives yields of over 90% for each cycle.

Calcium nitrate required for boron-deficiency studies is obtained by allowing a thin cream prepared from calcium carbonate and distilled water to react with the theoretical equivalent amount of nitric acid. Analytical reagent or " Batch Analysis " grade concentrated nitric acid is diluted 1 : 1 with distilled water in a 3-L. flask. The calcium carbonate used is either of analytical reagent grade or B.D.H. " Fluorine Free " grade (prepared by an ammonium carbonate precipitation). The thin cream is poured slowly into the acid which reacts without excessive effervescence. At the *end* of the reaction considerable frothing occurs and basic salts of iron and other elements are precipitated. The reaction is complete when a slight excess of solid calcium carbonate remains. This is filtered off on a Buchner funnel with a No. 42 paper and the volume made up to two litres.

The precise weights of carbonate and acid required should be calculated as follows :—

$CaCO_3$; (100 g) + $2HNO_3$ ($2 \times 63 = 126$ g)
$= Ca(NO_3)_2$ (164 g)

$\therefore$ 2 litres of 2M. $Ca(NO_3)$ (32·8%) require 400 g. $CaCO_3$ and 504 g. of HNO_3.

Suppose the nitric acid concentration is x% (e.g. 70%) and the acid has a specific gravity of y (e.g. 1·42), then the volume of acid required is

$$\frac{504}{y} \times \frac{100}{x} \text{ ml.}$$

in actual practice $\frac{100}{x.y}$ is usually almost unity.

In order to ensure complete neutralisation of the acid a slight excess of calcium carbonate should be used.

The salts prepared as described have been found to contain less than one part per million of boron in the solid salt. This concentration produces a boron supplement of less than 0·001 p.p.m. in the diluted nutrient solution.

Purification of Stock Solutions from Molybdenum

The method first employed at Long Ashton was based on the use of 8 hydroxyquinoline and iron to co-precipitate molybdenum.

In more recent work by Hewitt and Jones (1950, and unpublished) and Hewitt and Hallas (1951), copper sulphide has been used to co-precipitate molybdenum, as this method appears slightly more efficient (Table 18) than the use of 8 hydroxyquinoline and is simple to carry out. Both methods are given here :

PLATE H

Molybdenum deficiency in lettuce.
Left : **Without molybdenum.** *Right :* **Control.**

[*To face page* 193

(a) *Co-precipitation of molybdenum with 8 hydroxyquinoline.* Stock solutions of the macronutrient salts are prepared by dissolving separately the requisite weights of previously recrystallised analytical reagent or " batch analysis " grade salts (see under boron above) for 2 L. of solution (as given in Table 23), each in about 1,700 ml. of glass-distilled water in a 2-L. beaker. Sufficient redistilled hydrochloric acid is added to reduce the pH of the solution to 1·8 as determined by a glass electrode and pH meter. 56 mg. of iron as ferric chloride and 455 mg. 8 hydroxyquinoline (analytical reagent grade) which corresponds to a 5% excess of the equivalent amount required to precipitate the iron, are also added. 2N analytical reagent grade potassium hydroxide is run in slowly from a burette while the solution is stirred mechanically until the solution has a pH of 5 (pH values above 7 cause desorption and liberation of the molybdenum). The precipitate is allowed to settle overnight and the solution filtered through a No. 42 paper on a Buchner funnel. The filtrate is then extracted in a 4-L. separating funnel, once with a 1% solution of 8 hydroxyquinoline in redistilled chloroform, and then with chloroform only until the chloroform layer is colourless and the aqueous layer is colourless (or pale yellow in the case of calcium nitrate). The solution is finally extracted twice with redistilled ether. This final extraction improves the separation of the phases and removes the last traces of chloroform. The extracted solutions are set aside in a warm atmosphere before making up to volume directly in calibrated standard 2-L. pyrex glass-stoppered bottles.

(b) *Co-precipitation with copper sulphide.* Copper sulphide is one of the most efficient of a number of sulphides that have been tested for the removal of molybdenum and avoids the introduction of a non-essential element. On the other hand, the antagonism of copper and molybdenum recognised in animal (or microorganism ?) nutrition implies that caution may be needed when effects of extremely low concentrations of molybdenum are studied. Unpublished work has shown that certain heavy metals may interfere with the utilisation of molybdenum at low concentrations. Cadmium sulphide alone is not effective (Table 18) and its use is toxic to *A. niger*. Cadmium plus copper and lead plus copper sulphides are not significantly more efficient than copper alone and for higher plants their use is unnecessary. The method worked out at Long Ashton and described below was based on the reported use of copper sulphide by Stout and Meagher (1948).

The Molar or 2-Molar stock solutions of already recrystallised analytical reagent grade salts are prepared in 3-L. pyrex or hysil glass beakers. 10 ml. of 19·5% $CuSO_4\ 5H_2O$ are added per litre of stock solution and stirred. (A few drops of redistilled hydrochloric acid are added to produce a pH of 2-4, but this is probably not essential.) Hydrogen sulphide from a freshly set up and clean Kipp's apparatus is passed into the solution. The gas is filtered through cotton wool and a spray trap. The gas is passed for 30 minutes for most salts and for 60 minutes for calcium salts and phosphates. The precipitate is filtered through a No. 42 paper on a Buchner funnel kept solely for this work, or through a No. 4 pyrex glass sinter funnel (cleaned with hot hydrochloric-nitric acids).

A portion of the filtrate is tested with more hydrogen sulphide to ensure complete precipitation and if necessary the treatment is repeated. This is only rarely necessary. The solution is simmered on a hot plate (and protected from dust) to remove the residual hydrogen sulphide and precipitate colloidal sulphides and sulphur and is then refiltered after it has been left to cool and stand for 1-2 hours. The solution is made up to volume in the calibrated pyrex stock bottles.

Extraction with purified dithizone solution (that has also been extracted several times with glass-distilled water) will remove the last traces of copper, if this is required for simultaneous work on copper nutrition, without the reintroduction of molybdenum.

The procedure described here has an efficiency of more than 99·9% for the removal of molybdenum and a single operation is usually completely satisfactory for the preparation of solutions for use with molybdenum deficiency tests in higher plants.

The Purification of Compounds used to Supply Iron

The compounds used to supply iron may contain significant amounts of several micronutrients and these should be removed. The procedure used may depend on the purpose for which the iron salt is needed and methods are given here for (i) the removal of manganese, copper and zinc as one group ; (ii) the removal of molybdenum, and (iii) a tentative method for the preparation of an iron compound free from the elements stated and also from gallium.

(i) The Removal of Manganese, Copper and Zinc

The method for the elimination of manganese, copper and zinc is that originally recommended by Piper with slight modification. This method is based on the differential solubilities of ferric chloride as compared with those of zinc, copper and manganese in ether in the presence of 6N hydrochloric acid. The procedure used is as follows (after Piper, private communication) :—Dissolve 500 g. analytical reagent grade ferric ammonium sulphate in 350 ml. hot, glass-distilled water and filter through a No. 44 paper. Transfer filtrate to a 2-L. pyrex separating funnel and add 1,250 ml. concentrated analytical reagent grade hydrochloric acid saturated with ether (prepared as described below). Shake well (with care to release pressure frequently) and run off the (lower) aqueous phase into a second funnel. Add 400 ml. redistilled ether to the latter and extract again ; combine the ether fractions. Wash the ether fraction with 5 successive 200 ml. portions of *redistilled* hydrochloric acid saturated with ether (prepared as described below) and discard the washings. Shake the ether fraction with 5 successive 200 ml. portions of glass-distilled water and combine these in a pyrex glass reagent bottle for storage.

Determine the iron content in a 5 ml. aliquot precipitated by ammonia. Convert ferric chloride to citrate as follows :—Transfer the equivalent of 16·25 g. $FeCl_3$ $6H_2O$ to a clean silica evaporating dish, and add 20 g. recrystallised analytical reagent grade citric acid. Evaporate on a boiling water bath (provided with glass or porcelain rings) for 8-12 hours with frequent stirring until the mass is nearly solid. Redissolve in glass-distilled water and make up to 1 L. This solution contains 5·6 mg. *ferric* iron per ml.

(a) Concentrated hydrochloric acid saturated with ether is prepared by the addition of 200 ml. portions of redistilled ether to 700 ml. concentrated analytical reagent grade acid cooled in ice until an ether layer persists.

(b) Redistilled hydrochloric acid saturated with ether is prepared by the addition of redistilled ether to 800 ml. of ice-cooled redistilled acid (S.G. 1·10 or 20·2% concentration) until an ether layer persists.

(ii) The Removal of Molybdenum

Molybdenum is extracted into the ether phase together with ferric iron and cannot be separated from it in the manner described above. Molybdenum forms a complex with dithiol which is soluble in amyl alcohol in the presence of hydrochloric acid as stated in Part One. Molybdenum can thus be conveniently removed by extraction of the acid ferric chloride solution obtained in the method above, with a *fresh* 1·0% solution of dithiol in amyl acetate from a newly opened ampoule. The molybdenum complex produces a green colour. The acid concentration in the aqueous phase should be 15%. The ferric chloride is converted to citrate as described in (i) above, but the analytical reagent grade citric acid is first twice recrystallised from glass-distilled water and may also be purified by the copper sulphide precipitation method. This method was used by Hewitt and Jones (1947) (1949).

(iii) Tentative Method for the Preparation of Iron Compounds Free from Manganese, Copper, Zinc, Molybdenum and Gallium

This method has been developed as part of a project to study methods for the preparation of solutions as free as possible from several micronutrients simultaneously to facilitate micronutrient interaction studies. It has been used by Hewitt and Jones (1950) and others at Long Ashton in molybdenum deficiency studies.

Analytical reagent grade ferrous ammonium sulphate is used as the starting point in this process. One kilogram of this salt is first recrystallised from glass-distilled water (in the presence of 1% sulphuric acid to repress hydrolysis and with the addition of an equal volume of redistilled alcohol to enhance the yield). The crystals are separated on a Buchner funnel, washed in alcohol and redissolved in glass-distilled water to give an almost saturated solution at room temperature. The pH is reduced to 2·8 with pure hydrochloric acid and the solution is repeatedly extracted with an 0·5% solution of 8 hydroxyquinoline in chloroform and finally with chloroform only. This extraction causes molybdenum and gallium compounds to pass into the chloroform phase while ferrous iron compounds remain in the aqueous phase, *cf.* Sandell (1944) and Welcher (1947). The black coloration is due to a trace of ferric iron which reacts with the 8 hydroxyquinoline. The extractions are completed with chloroform only, until colourless, to remove the last traces of 8 hydroxyquinoline. The aqueous phase is now mixed with 10 N. analytical reagent grade hydrochloric acid to produce an acid concentration of 6 N. and carefully saturated with ether. Excess ether is added and the aqueous phase is extracted with 5 successive 200 ml. portions of ether. This extraction is particularly efficient in the removal of gallium according to Sandell (1944) and also removes molybdenum. A precipitate may appear at this stage and the volume of 6 N. acid may require to be increased.

The ferrous iron is converted to the ferric form by addition of hydrogen peroxide. The peroxide is first distilled under reduced pressure (with care) in an all-pyrex glass assembly with ground glass joints, as no rubber bungs or corks are permissible. The amount of hydrogen peroxide required is determined by the titration of an aliquot with a permanganate solution which is also used to titrate with an aliquot of the ferrous salt solution, in dilute solution to prevent interference from the hydrochloric acid. Approximately 1% of the ferrous iron should be left in excess and dipyridyl is a suitable external indicator. The ferric chloride solution thus obtained is evaporated nearly to dryness and redissolved in 500 ml. redistilled 6 N. hydrochloric acid and saturated with redistilled ether. The solution is extracted three times with redistilled ether and the ether fractions are retained. The ether phase is then shaken with 3 successive 100 ml. portions of 6 N. redistilled hydrochloric acid saturated with ether. These aqueous fractions are discarded. The ether fraction is then shaken with 5 successive 100 ml. portions of glass-distilled water to re-extract the ferric chloride into the aqueous phase. Ferric citrate is then prepared as already described, but the citric acid should not be treated with copper sulphide, or else it must be converted to ammonium citrate and extracted with dithizone (pH 3-4).

This procedure is lengthy; two separate stages are instrumental in the removal of molybdenum and gallium, and one in the removal of copper, zinc, manganese and probably several other elements.

The Preparation of Manganese Compounds Free from Iron or Molybdenum

Manganese compounds may be required (particularly in high concentrations) for use in interaction with iron or molybdenum.

Removal of Iron

This method is based on the details given by Partington (1933, p. 944). Dissolve 0·84 g. sodium bicarbonate in a 24·5% solution of manganese sulphate and boil. The precipitate is removed by filtration through a No. 42 paper on a Buchner funnel. The manganese concentration is determined colorimetrically with potassium periodate and adjusted to a convenient concentration, e.g. 22·5% or 2·25%. (See Table 23.)

Removal of Molybdenum

The 8-hydroxyquinoline precipitation (a), ether-hydrochloric acid extraction (b), or copper sulphide (c) methods are suggested for this purpose.

(a) A solution containing 22.5 g. of manganese sulphate in 800 ml. glass-distilled water is brought to a pH of 1·8 with hydrochloric acid and 56 mg. of iron as ferric chloride and

500 mg. of 8 hydroxyquinoline are added, and dissolved. The pH is raised to 5 and the precipitate so produced is left to stand overnight. The solution is filtered and extracted as described for the purification of the macronutrient salts required for molybdenum deficiency. The volume is adjusted to give a convenient concentration, e.g. 2·25%.

(b) This second method has not been tested, but ether extraction of manganese sulphate (2-5% concentration) in the presence of redistilled 6 N. hydrochloric acid saturated with ether and a few drops of hydrogen peroxide should be effective in the removal of iron and molybdenum. The ether fractions are discarded and the manganese salt obtained by evaporation until crystallisation takes place.

(c) Co-precipitation with copper sulphide, as described above.

Cleaning of Glassware and Other Apparatus

All glassware and Buchner funnels used for the procedures given here (except molybdenum deficiency) are cleaned first in hot soap and water, hot 1 : 1 hydrochloric acid and then rinsed with ordinary and finally glass-distilled water. Reagent bottles used to store the purified solutions are cleaned as described and deposits are rapidly removed by shaking paper pulp in the 1 : 1 acid, but in addition each bottle is steamed for at least one hour with the vapour from a constant boiling point hydrochloric acid solution before rinsing with distilled water. All glassware used for work in connection with deficiencies of iron, manganese, copper, zinc, molybdenum, gallium, etc., is of pyrex or hysil glass. Solutions required for boron deficiency are stored in soft glass bottles. All glassware used for molybdenum deficiency is also steamed with the vapour of boiling 20% hydrochloric acid before rinsing several times with glass-distilled water.

Testing Nutrient Solutions

Purified nutrient solutions are tested to ensure that the batch to be used is satisfactory for the purpose required, and especially molybdenum, p. 68 and Table 18.

Test for Iron

Purified potassium thiocyanate : Dissolve 100 g. KCNS in about 450 ml. distilled water. Add 5 ml. hydrochloric acid and extract repeatedly with iron-free amyl alcohol until no pink colour is detected. Allow to stand and separate completely. Make up to 500 ml. with glass-distilled water and store in the dark.

Introduce 180 ml. of stock solution into a stoppered cylinder or volumetric flask, add 2 ml. of redistilled hydrochloric acid, 5 ml. of purified KCNS, 10 ml. iron-free amyl alcohol, and shake vigorously. Allow to separate and compare the pink colour with standards prepared similarly, or determine the iron content colorimetrically. Reagents prepared by purification with the adsorption method usually give a scarcely detectible pink colour.

Test for Manganese

Proceed with stock solutions as described in section on water supply.

Test for Copper, Zinc and Several Heavy Metals

Proceed with stock solutions as described in Section on water. No pink colour should be produced.

Test for Boron

The method is based on that of Scharrer and Gotschall (1935).

The solid salt (10 g.) is weighed into a 100 ml. silica round bottom flask with a ground joint fitted to a reflux condensor (all in silica). 20 ml. concentrated (analytical reagent grade) sulphuric acid, 5 ml. water and 25 ml. pure (redistilled from potash) methyl alcohol are added and the mixture refluxed gently for one hour. The alcohol is then distilled off into 5 ml. 1% solution of recrystallised potassium carbonate to fix the boron. A second 25 ml. portion of

methyl alcohol is added to the flask and distilled off into the potassium carbonate. The alcohol is removed by gentle evaporation. The residue is slightly acidified with a few drops of sulphuric acid and boiled for two minutes with hydrazine sulphate. This is essential to decompose any nitrate present which otherwise causes a high result. The solution is then made up to a volume of 5-25 ml. according to the probable boron content and the boron is determined colorimetrically with quinalizarin as follows. Small stoppered bottles or tubes of 25 ml. capacity are first charged with 9 ml. of 98% analytical reagent grade sulphuric acid, 1 ml. of an 0·01% W/V solution of quinalizarin in 93% sulphuric acid and mixed by shaking. *The stoppers are replaced between all operations.* 1 ml. of the test solution is added and the vessel stoppered and shaken. It is left to cool and stand at least 6 hours. The boron content is determined from the drum reading on the Spekker absorptiometer (red filters) with the aid of a calibration curve, covering the range 0·0-0·01 mg. B/ml. or from a series of standards similarly prepared with a standard solution of boric acid. 0·5 p.p.m. B in solid reagents can be detected.

General Technique

This section deals with layout, seed raising, methods of watering, pest control, choice of crop, etc.

Layout and Treatment Code

The pots are arranged in double rows on slatted trestle benches about 12-18 inches above the ground and these run North to South across the width of the culture house. About 30 pots can be accommodated in each row. Until recently the layout has been systematic in design for two reasons : to facilitate recording of visual symptoms and to avoid errors in watering, as 1,200 or more pots require attention daily and with up to fifty separate treatments the risk of mistakes was considered too great. Experience has shown since, however, that mistakes may occur more readily if several treatments that relate to adjacent small groups of pots in close contact are arranged systematically than if the treatments are distributed at random in such a way that each must be found separately. The major difficulty of locating each treatment rapidly and reliably at a distance has been overcome by the use of a sign code painted on the sides of the pots or on cardboard placards in bright colours, and on the corresponding bottles of nutrient solutions. This method is preferred to others involving numerical codes, or letters with a numerical *suffix* ; treatments are easily memorised and less likely to be confused. The precise nature of the treatment can be easily discerned when a large number of factorial combinations are involved. As far as possible the basic treatments are shown by distinctive, simple, geometrical, solid outlines and these are replicated when used in factorial experiments with more than one level. An open outline indicates deficiency of the element depicted. As many experiments at Long Ashton have involved the study of iron supply the colour code has been reserved mainly for variations in the level of this element. Thus four levels of iron from very low, to very high, are shown by yellow, white, pale blue and green respectively. Source of nitrogen is indicated by the addition of a background colour, for example red, to indicate ammonia or urea in place of nitrate. White is used for all outlines to indicate that the standard levels of iron and nitrogen as nitrate are present. The complete nutrient solution is always shown as a solid circle when used in a deficiency series.

This scheme allows a clear presentation of treatment and provides sufficient scope to indicate treatments varying simultaneously with respect to source of nitrogen, and up to 3 levels of N, P, K, Ca or Mg, level of Mn or Al, level of Fe and level of Mo. Extensions of this scheme may be readily devised to accommodate other requirements, and factorial combinations of 64 treatments can be easily accommodated.

Raising Experimental Plants

The method used for seed raising depends upon the nature of the experiments to be made. The general principles affecting the choice for culture work have been discussed. Certain seeds may be frequently sown directly in the sand with advantage. These include seeds of

cereals and grasses, beans, peas and clovers, globe and sugar beet, mangold, flax and linseed, carrot, parsnip, swede, turnip, mustard, radish, celery and lettuce. The seedlings are thinned, preferably twice at suitable stages, for uniformity and vigour. Beans and peas are sown about 2 inches deep, cereals an inch deep and small seeds about one half-inch deep. A 10-inch pot will support 3 pea plants or 2 bean plants, 5 clovers, 10 cereal plants and 3 swede, turnip or beet, etc.

Some plants are usually raised either in sand or standard John Innes compost as described by Lawrence and Newall (1941) (with reduced peat to avoid penetration of peaty fragments by fine roots) and transplanted when required at a suitable stage according to the size of the plant preferred. These plants include most Brassicas, tomato and lettuce ; usually only one seedling is transplanted.

Seedlings are raised in standard compost when required for work on such problems as the resolution of factors in acid soils, toxicity treatments, studies of deficiency symptoms of the macronutrients ; such seedlings can also be used successfully for work on iron and boron deficiencies. The seedlings are removed carefully from the well-moistened soil with a ball of soil and the main mass is removed by gently raising and lowering the plant's roots in a bucket of clean rain water. Small particles are then removed carefully by hand and the seedlings are rinsed in a second bucket of clean water. The seedlings are left standing in clean water until sufficient are ready to plant a convenient batch of pots. Seedlings are easily grown in purified sand for the most exacting work and it is then necessary to supply them with appropriately prepared nutrient solution. They may be transplanted without the added interference of root washing.

Method of Planting

The seedlings are planted by making a wide slit-like hole in the sand for tap-rooted plants or seedlings. The roots are allowed to spread out in water and are maintained spaced out by lifting them out against the flat of the hand. The roots are laid out against one side of the slit and the sand gently pressed in against them. The sand is then watered with the appropriate solution and the pot is gently rocked to settle the sand around the roots and to level the surface. When seedlings are transplanted at the cotyledon stage before the true leaves have appeared, as with Brassicas and tomato used for molybdenum-deficiency experiments, 2 or more are usually transplanted into each pot into small holes made with a glass rod and then watered in. The seedlings are gently eased out of the sand seed-bed with a glass rod and the ball of sand is allowed to drop away by immersion in distilled water. They are thinned out to one as soon as it is obvious that the seedlings have " taken " and are apparently normal (about 7 days).

Potato tubers are not cut. Tubers weighing about 60 g. are usually chosen and selected from well-sprouted tubers. Certified Grade A Scotch seed should be used.* All but two or three shoots are removed and the tubers are planted 4 inches deep.

Treatment of Sand at Planting Time

The solution applied to the sand to saturate it before planting depends on the nature of the experiment. For the production of plants to demonstrate deficiency symptoms of nitrogen, phosphorus, potassium or magnesium respectively with increasing severity during growth, a half or one litre of complete nutrient may be given before planting, followed by the appropriate deficiency solutions. Experience has shown that phosphorus, magnesium and often potassium should be omitted from the outset with cereals and no extra magnesium given to beet. Calcium-deficiency effects may not be produced readily until a late stage if an initial quantity of calcium is given, especially to unleached sand. Complete lack of calcium in leached sand, however, may cause early death and a level of 0·25 m.eq./L. (5 p.p.m.) is a suitable deficiency level for Brassicas ; more may have to be given for potato in the initial stages and

* Specially selected virus-free Majestic stock tubers have been supplied annually recently through the courtesy of Francis K. Balfour, Esq., Blairgowrie, Perth, and have proved highly satisfactory for culture experiments.

PLATE I

Molybdenum deficiency in kale.
Left : **Without molybdenum.** *Right :* **Control.**

To face page 199

less for other crops. No additional boron, manganese or iron is given when acute deficiency effects are to be produced. The efficiency of the methods given here may be judged by observing the time required to produce *typical* symptoms of deficiencies of iron, boron, manganese or molybdenum. A few examples of representative times for test plants will serve as guides for deciding whether the technique used has been effective :—

Boron deficiency : tomato, cauliflower, flax, beet, clovers : 7-10 days from emergence or transplanting.

Iron deficiency : oat, 10 days ;
tomato, sugar beet, 10-14 days.

Manganese deficiency : oat, tomato, sugar beet, 10-14 days.

Molybdenum deficiency : swede, cauliflower, tomato, lettuce, 20 days.

Examples of representative effects are shown in Plates B, C, F, G, H & I.

Change of Treatment

When it is necessary to change a treatment during growth of the plant so that the level of a particular macronutrient element is greatly lowered, the sand is leached with 4 successive portions of 1 L. of water followed by 2 L. of the new nutrient solution. The procedure has not been tested for micronutrients. Experience of others reviewed in Part One suggests that such treatment may be suitable for boron deficiency.

Frequency of Watering

The cultures are normally watered according to requirements. These depend on the time of year, weather and size of plants. The maximum amount of solution required rarely exceeds 1 L. daily for vegetables and may be only 0·5 L. on alternate days. The requirement is judged by experience and over-watering is avoided. In some experiments it may be desirable to give all cultures the same volume of solution, but usually the volume given is roughly proportional to the growth. The state of the sand is easily determined by testing the surface with a glass rod reserved for use in cultures receiving the same treatment. By this means it can be seen whether the sand is saturated, just drying out and " crumbling," or powdery on the surface.

The solution is applied to the surface of the sand with a circular motion to distribute the liquid around the periphery of the pot. This helps to avoid root disturbance or exposure. The service tanks are kept filled overnight in order to allow water to come to the temperature of the house before application and so that watering is not delayed by refilling the tanks during the morning. Distilled water used for molybdenum-deficiency or other highly refined techniques is stored and transported in 50-litre pyrex glass carboys held in wicker baskets. The water is removed by siphoning through wide-bore glass tubing. The siphon is kept filled and controlled by a specially designed clip already described or a wide-bore (12 mm.) glass stopcock. The rubber tubing is boiled first in dilute hydrochloric acid and then distilled water. All glassware, including siphon, measures and carboys, is cleaned with hot 1 : 1 acid and steamed before rinsing with distilled water as described. Enamel measures (2 L. and 1 L.) are used for macronutrient experiments. Pyrex glass beakers (1,300 ml.) or 5 L. pyrex bottles with glass pourers are used to distribute solution in micronutrient experiments.

Pest Control

Pest control measures are limited by the fact that sprays are often an easy means of applying a micronutrient to a plant. The difficulties in the preparation of a pure spray (including the wetting agent) make spraying difficult when micronutrient-deficiency cultures are affected. Nicotine can be distilled and dissolved in glass-distilled water (0·05%). A wetting agent can be prepared by distillation of oleic acid *in vacuo* and triethanolamine, which are then mixed to give the ester* which has useful wetting properties as a 1% solution. This spray mixture has been used to control aphis attacks on cultures lacking iron, manganese and boron. E. W. Jones (unpublished) and S. C. Agarwala (unpublished) used an aqueous (glass-distilled

* This was prepared for the writer by Dr. D. Woodcock on the suggestion of Dr. H. Martin, both of Long Ashton, and their assistance is gratefully acknowledged.

water) suspension of D.D.T. recrystallised from distilled acetone to obtain some measure of control of aphides on potatoes and cauliflowers respectively, without interference in micronutrient deficiency studies. Plants in macronutrient-deficiency cultures can be sprayed with 0·05%-0·1% nicotine with ester salts as a wetting agent. Brassicas are liable to show nicotine injury (a white rim around the expanded leaves) when sprayed frequently at 0·1% concentrations, or if excessive wetting agent is present. The nicotine spray is conveniently prepared by mixing one part of pure nicotine with two parts water. 15 ml. of this mixture diluted to 5 L. gives a concentration of 0·1%. " Ester salts " is added at the rate of 0·5 fluid oz. per gallon or 15 ml. in 5 L. of spray. Methyl cellulose has also been used as a wetting agent to reduce contamination. Mildew in cereals and clover is controlled by applying weekly a colloidal sulphur spray containing " sulphite lye " as a wetting agent. No effective control for mildew in micronutrient-deficiency cultures has been devised. Fumigation and systemic compounds have not been used as their interaction with mineral disorders is still unknown.

Choice of Crop

The choice of crop plant used in these culture experiments is determined by several factors ; a few examples may be given. The tomato is usually grown when a new aspect of technique is examined since its reaction to nutritional conditions is well known, and it has moderately high requirements for most nutrient elements. Tomato, potato, cereals, sugar beet and beans are useful in the study of problems of iron and manganese metabolism. Brassicas, tomato, beet, celery, lettuce and legumes respond readily to boron deficiency and are also of special interest in relation to molybdenum nutrition. Cereals are " difficult " subjects for boron-deficiency and may be used to test greater refinements in technique. In general, however, their susceptibility to severe attack by mildew is a disadvantage. Tomato, potato and leguminous types are useful types for study of potassium, phosphorus, magnesium and calcium deficiencies. Cereals, tomato, lucerne and red clover are good subjects for copper and zinc deficiency tests. Potato, tomato, sugar beet and cereals are useful types for the study of yield data. Carrot, sugar beet, celery, potato and beans are suitable for studies on aluminium toxicity and Brassicas, beans and potato give interesting effects with excess of manganese. Sugar beet is especially suitable for experiments on heavy metal—iron interrelationships.

The results obtained during the development of the Long Ashton sand cultures have shown beyond doubt that the large scale sand culture methods are entirely satisfactory as a means for studying all known micronutrient deficiencies and interactions of these elements. Ion-exchange water treatment appears able to overcome most of the difficulties of a pure water supply for large scale work. The general standard achieved is reproducible and not less efficient than that claimed for other published techniques including water culture methods on a comparable scale.

ACKNOWLEDGMENTS

This Technical Communication was suggested by Professor T. Wallace, C.B.E. and owes much to his interest and encouragement. Mr. D. Akenhead, O.B.E., Director, and Mr. G. K. Argles, Assistant Director of the Commonwealth Bureau of Horticulture gave invaluable assistance in procuring papers and translations, and in many other respects.

Sir Edward Salisbury, C.B.E., Vice-President of the Royal Society, Director of the Royal Botanic Gardens, Kew, has kindly consented to provide a foreword.

Reprints and Abstracts were provided with the help of the Official Correspondents of the Commonwealth Bureau : Mr. Frank Read (Canberra), Mr. M. B. Davis (Ottawa) and the Director of the Indian Council of Agricultural Research, and personal assistance was most generously given by the Librarians of the Royal Society and the University of Reading and the Librarians of the East Malling and Long Ashton Research Stations.

Extensive personal communications, unpublished data, or permission to reproduce illustrations were kindly given by the following :

Professor D. I. Arnon, Professor L. J. Audus, Mr. R. S. Beckwith, Dr. B. D. Bolas, Dr. W. E. Brenchley, O.B.E., Professor H. D. Chapman, Dr. E. B. Davies, Dr. F. M. Eaton, Dr. H. Evans, Professor H. G. Gauch, Mr. M. Greenwood, Professor F. G. Gregory, F.R.S., Dr. E. C. Humphries, Professor A. L. Kenworthy, Dr. J. J. Lehr, Professor E. J. Maskell, F.R.S., Dr. E. P. Mehrlich, Dr. C. R. Millikan, Dr. H. L. Pearse, Dr. C. S. Piper, Mr. F. J. Richards, Mr. H. C. Ruck, Professor J. W. Shive, Professor H. C. Trumble, Professor E. S. Twyman and Dr. K. Warington, whose co-operation and goodwill are sincerely appreciated.

Assistance in the design or construction of equipment described in Part Two was given by Mr. E. W. Bolle-Jones, Dr. H. G. H. Kearns and Mr. F. E. Maltby, all of Long Ashton, Mr. E. J. Winter of the National Vegetable Research Station, and Mr. J. H. Burrow of the University of Bristol ; the photographs are by Mr. G. H. Jones of Long Ashton, to all of whom sincere thanks are offered.

The illustrations in Figures 1, 3 A—C, 5 A—H, and 8 were expertly re-drawn by Miss M. Durling of the Commonwealth Bureau and by Mr. E. Y. Jones and Miss J. C. Irving of East Malling Research Station, whose help is gratefully acknowledged. The block of Figure 7 A was presented by the Chronica Botanica Co. through the kindness of Dr. F. Verdoorn and the drawing of 7 B was made and kindly presented by the Kestner Evaporator and Engineering Company. Permission to reproduce or re-draw text figure illustrations was generously given by the Editors and Publishers of the following Journals :

East Malling Research Station Annual Report for figure 6 E ;
Industrial and Engineering Chemistry (Analytical Edition) for figure 2 ;
The Journal of Experimental Botany and the Clarendon Press, Oxford for figure 5 K ;
Plant Physiology for figures 5 E, 6 B, C ;
Science for figures 5 B, G, H, I.

Finally, it is a pleasure to state that this Communication as a whole, and the techniques described in Part Two in particular are the outcome of work sponsored during the last nine years by the Agricultural Research Council whose generous support is most gratefully acknowledged.

E. J. Hewitt,
December, 1951. Long Ashton Research Station.

BIBLIOGRAPHY AND AUTHOR INDEX

(*Prefix* T *signifies Table number*)

ADAMS, B. A., and HOLMES, E. L. (1935). *J. Soc. chem. Ind. Lond.* **54** (Transactions) : 1. Absorptive properties of synthetic resins. 59

ADAMS, F. W. (1935). *J. Soc. Glass. Tech.* **19** (Transactions) : 119. A new process for the removal of iron oxide from silica sands. 31, 175

ADDOMS, R. M. (1937). *Plant Physiol.* **12** : 199. Nutritional studies on Loblolly pine. 9, 21, 96, T19B, 119, 143

ADDOMS, R. M., see NIGHTINGALE, G. T.

AGARWALA, S. C. (1951). *A.R. Long Ashton agric. hort. Res. Stat.* 1950, p. 83 (and unpublished data). The effect of molybdenum and nitrate status on yield and ascorbic acid content of cauliflower plants in sand culture. 114, 191, 199

AGARWALA, S. C., see HEWITT, E. J.

AGHULON, H. (1910) (quoted by Miller 1938). Recherches sur la présence et le rôle du bore chez les végétaux. Thèse (Paris). 3

AHI, S. M., and POWERS, W. L. (1938). *Plant Physiol.* **13** : 767. Salt tolerance of plants at various temperatures. 91, 160

AHLGREN, G. H. (1941). *Soil Sci.* **52** : 229. Some effects of volume, rate of solution supply and potassium concentration on the growth of white clover. 84, 87

AHLGREN, G. H. (1942). *Bull. New Jersey agric. Exp. Stat.* **692**. Influence of thiamin additions on germination and growth of certain grasses and white clover. 115

ALBEN, A. O., HAMMAR, H. E. and SITTON, B. G. (1942). *Proc. Amer. Soc. hort. Sci.* **41** : 53. Some nutrient deficiency symptoms of the pecan. 8, 30, 96, T19B, 118, 143

ALBERT, A., and GLEDHILL, W. S. (1947). *Biochem. J.* **41** : 529. The choice of a chelating agent for inactivating trace metals. I. A survey of commercially available chelating agents. 80

ALBERT, A., and MCGRATH, D. (1947). *Biochem. J.* **41** : 534. The choice of a chelating agent for inactivating trace metals. 2. Derivatives of oxine (8-hydroxyquinoline). 80

ALBRECHT, W. A. (1932). *J. Amer. Soc. Agron.* **24** : 793. Calcium and hydrogen ion concentration in the growth and nodulation of soya beans. 38

ALBRECHT, W. A. (1946). *Soil Sci.* **62** : 23. Preparation of the clay and procedures in its use as a plant growth medium. 35

ALBRECHT, W. A., and MCCALLA, T. M. (1938). *Amer. J. Bot.* **25** : 403. The colloidal clay fractions of soil as a cultural medium. 38

ALBRECHT, W. A., see GRAHAM, E. R.; WITTWER, S. H.

ALLISON, F. E., see HORNER, C. K.

ALLISON, R. V. (1921), *A.R. New Jersey agric. Exp. Stat.* 1920, p. 338. The relation of aeration to the development of the soya bean plant in artificial culture. 127, 135, 154

ALLISON, R. V. (1923). *A.R. New Jersey agric. Exp. Stat.* 1922, p. 402. Effect of aeration and continuous renewal of solutions upon the growth of barley and buckwheat in artificial culture. 127, 135, 154

ALLISON, R. V., and SHIVE, J. W. (1923). *Amer. J. Bot.* **10** : 554. Studies on the relation of aeration and continuous renewal of nutrient solutions to the growth of soya bean in artificial culture. 120, 124, 125, 129, 133, 135

ALSTINE, E. V. (1920). *A.R. New Jersey agric. Exp. Stat.* 1919, p. 395. The interrelation between plant growth and the acidity of nutrient solutions. 98, 103

Analyst (Anon) (1944). *Analyst,* **69** : 243. Report of sub-committee on fluorine in foodstuffs. 71

ANDERSON, A. J. (1944a). *J. Coun. sci. industr. Res. Aust.* **17** : 144. Double compartment pot cultures for studies in plant nutrition. 24, 25

ANDERSON, A. J. (1944b). *J. Coun. sci. industr. Res. Aust.* **17** : 151. An automatic irrigator actuated by a soil moisture tensiometer. 124

ANDERSSEN, F. G. (1926). *Plant Physiol.* **1** : 417. A device for maintaining constant level of culture solutions. 122

ANDREWS, F. M. (1919). *Proc. Indiana Acad. Sci.*, p. 165. Apparatus for aerating plants. 135

ANDREWS, F. M., and BEAL, C. C. (1919). *Bull. Torrey bot. Club* **46** : 91. Effect of soaking in water and of aeration on the growth of *Zea mais.* 133

APPLEMAN, C. O. (1918). *Bull. Md. agric. Exp. Stat.* **212**, p. 79. Physiological basis for the preparation of potatoes from seed. 154, 164

ARCHIBALD, E. H. (1932). " The preparation of pure inorganic substances." John Wiley, New York. 70, 82

ARENZ, B., see SCHROPP, W.

ARNDT, C. H. (1922). *Amer. J. Bot.* **9** : 47. The growth of field corn as affected by iron and aluminium salts. 155

ARNDT, TH., and HOFFMAN, W. (1937). *Landw. Vers Sta.* **129** : 71. Spurenelemente und ihre Wirkung auf das Pflanzenwachstum unter besonderer Berücksichtigung von Versuchsergebnissen mit Kupfer. 22, 31, T7, 49, 51, 54, 55, 70, 80, 105, 109, 113, 151

ARNON, D. I. (Private communications). 52

ARNON, D. I. (1937). *Soil Sci.* **44** : 91. Ammonium and nitrate nitrogen nutrition of barley at different seasons in relation to hydrogen ion concentration, manganese, copper and oxygen supply. 12, 14, 94, T19B, 98, 99, 101, 110, 117, 133, 137, 159

ARNON, D. I. (1938). *Amer. J. Bot.* **25** : 322. Micro-elements in culture solution experiments with higher plants. 4, 17, 51, 85, 89, 96, T19A, 101, 103, 108, 109, 113, 114

ARNON, D. I. (1940). *Science* **92** : 264. Vitamin B_1 in relation to the growth of green plants. 37, T19B, 110, 113, 115

(ARNON, D. I.) (1948). *Nature*, **161** : 364-365. Trace elements in plant physiology. (Report of contributions to a symposium held at Rothamsted, November 1947). 1, 4

ARNON, D. I. (1950a). *Lotsya*, **3** : 31. Criteria of essentiality of inorganic micronutrients for plants with special reference to molybdenum. 4

ARNON, D. I. (1950b). *Lotsya*, **3** : 40. A memorandum regarding nomenclature. 1

ARNON, D. I., FRATSKE, W. E., and JOHNSON, C. M. (1942). *Plant Physiol.* **17** : 515. Hydrogen ion concentration in relation to absorption of inorganic nutrients by higher plants. 12, 14, 16, 98, 101, 117, 137, 139

ARNON, D. I., and FURNSTALL, A. H. (private communication). 52

ARNON, D. I., and GROSSENBACHER, K. A. (1947). *Soil Sci.* **63** : 159. Nutrient culture of crops with the use of synthetic ion-exchange materials. 8, 13, 19, 28, 32, 34, 35, 36, 37, 38, 57, 108, 114, 117, 131, 138, 144, 145

ARNON, D. I., and HOAGLAND, D. R. (1940). *Soil Sci.* **50** : 463. Crop production in artificial culture solutions and in soils with special reference to factors influencing yields and absorption of inorganic nutrients. 3, 6, 8, 13, 19, 37, 85, 88, 89, T19A, 98, 101, 131, 135, 139, 144, 145, 151, 154, 160

ARNON, D. I., and HOAGLAND, D. R. (1944). *Biol. Rev.* **19** : 55. The investigation of plant nutrition by artificial culture methods. 88

ARNON, D. I., and JOHNSON, C. M. (1942). *Plant Physiol.* **17** : 525. Influence of hydrogen ion concentration on the growth of higher plants under controlled conditions. 13, 14, 16, T19B, 98, 99, 101, 107, 108, 112, 114, 117, 133, 137, 151

ARNON, D. I., and MEAGHER, W. R. (1947). *Soil Sci.* **64** : 213. Factors influencing availability of plant nutrients from synthetic ion-exchange materials. 32, T7, 35, 36, 38, 39, 61, 141, 179

ARNON, D. I., and STOUT, P. R. (1939a). *Plant Physiol.* **14** : 371. The essentiality of certain elements in minute quantity for plants with special reference to copper. 4, 93, 158, 163, 164

ARNON, D. I., and STOUT, P. R. (1939b). *Plant Physiol.* **14** : 599. Molybdenum as an essential element for higher plants. 4, 22, 51, 67, 76, 108, 114

ARNON, D. I., STOUT, P. R., and SIPOS, F. (1940). *Amer. J. Bot.* **27** : 791. Radioactive phosphorus as an indicator of phosphorus absorption of tomato plants at various stages of development. 116

ARNON, D. I., see HOAGLAND, D. R., STOUT, P. R.

ARRINGTON, L. B., and SHIVE, J. W. (1936). *Soil Sci.* **42** : 341. Oxygen and carbon dioxide content of culture solutions in relation to cation and anion nitrogen absorption by tomato. 133, 139

ARROL, R. J. (1946). *Electronics Forum* No. 2, p. 8. Radioactive tracer technique and its application to biological research. 117

ASANA, R. D. (1945). *Indian J. agric. Sci.* **15** : 227. Observations on the influence of nitrate, ammonium and iron on the growth and nutrition of the rice plant. 16, 17, 21, 48, 95, T19B

ASCHOFF given by SCHROPP (1936). 106

ASHBY, E. (1929a). *Ann. Bot. Lond.* **43** : 332. The interaction of factors in the growth of *Lemna*. 3. The interrelationships of duration and intensity of light. 154, 157, 161

ASHBY, E. (1929b). *Ann. Bot. Lond.* **43** : 805. The interaction of factors in the growth of *Lemna*. 4. The influence of minute quantities of organic matter upon growth and reproduction. 115, 154

ASHBY, E., BOLAS, B. D. and HENDERSON, F. Y. (1928). *Ann. Bot. Lond.* **42** : 771. The interaction of factors in the growth of *Lemna*. 1. Methods and technique. 154, 157, 161

ATKIN, S., see JOHNSON, C. M.

AUDUS, L. J. (Private communication). 149

AUDUS, L. J., and QUASTEL, J. H. (1947). *Nature* **160** : 222. Toxic effects of some amino acids and amines on seedling growth. 98

AYERS, A. D., and HATCHER, J. T. (1939). *Proc. Soil Sci. Soc. Amer.* **4** : 314. Quantities of boron and zinc found in salts used in the preparation of culture solutions. 64, 65, 66, 68

AYERS, A. D., see JENNY, H. ; MAGISTAD, O. C.

AYRES, A. H. (1917). *Univ. Calif. Publ. agric. Sci.* **1** : 341. Influence of the composition and concentration of the nutrient solution on plants grown in sand cultures. 30, T7, 40

BAILEY, L. F., see HECK, W. W.

BAGULEY, A. (1912). *J. agric. Sci.* **4** : 318. The phosphate nutrition of plants. 41

BAIRD, K. J. (1951, private communication). 65

BAKKE, A. L., and ERDMAN, L. W. (1923). *Amer. J. Bot.* **10** : 18. A comparative stduy of sand and solution cultures for "Marquess" wheat. 28, 137, 154

BAKKER, E. M. VAN ZINDEREN. Private communication on composition of rain water. 44

BARKER, B. T. P. (1921). *A.R. Long Ashton agric. hort. Res. Stat.* 1920, p. 43. Root development in newly planted trees. 131, 154

BARKER, B. T. P. (1922). *A.R. Long Ashton agric. hort. Res. Stat.* 1921, p. 9. Studies on root development. 122, 141

BARKER, H. A., and BROYER, T. C. (1942). *Soil Sci.* **53** : 467. Notes on the influence of micro-organisms on growth of squash plants in water culture with particular reference to manganese nutrition. 110, 137, 149, 150

BARNETTE, R. M. (1921). *A.R. New Jersey agric. Exp. Stat.* 1920, p. 345. The influence of volume upon the rate of change in the hydrogen ion concentration of nutrient solutions in contact with plant roots and the effect of this change upon iron availability. 10, 14, 101, 104

BARNETTE, R. M., and SHIVE, J. W. (1923). *Soil Sci.* **15** : 413. The influence of solution volume upon plant growth in relation to reaction change and iron availability in culture solutions. 95, 103

BARTHOLOMEW, R. P., and JANSSEN, G. (1929). *Soil Sci.* **27** : 189. Relation between concentration of potassium in culture solutions and optimum plant growth. 11, 86

BARTHOLOMEW, R. P., see JANSSEN, G.

BASTISSE, E., see DEMOLON, A.

BATJER, L. P., and DEGMAN, E. S. (1940). *J. agric. Res.* **60** : 101. The effects of varying amounts of nitrogen, potassium and phosphorus on the growth and assimilation in young apple trees. 8, 21, 87, T19B, 119, 141

BATJER, L. P., see CULLINAN, F. P.

BAYLISS, L. E. (1938). *J. Mar. biol. Ass. U.K.* **23** : 99. The photographic method for recording average illumination. 163

BAYLISS, N. S., see SMITH, M. E.

BEAL, C. C., see ANDREWS, F. M.

BEAR, F. E., see EVANS, H. J.

BEARD, F. H. (1932) *J. Pomol.* **10** : 92. Manurial experiments with hops in pot culture. 5, 8, 15, 19

BEAUMONT, A. B. (1930). *Proc. 2nd Internat. Congr. of Soil Sci.* Commun. 4, p. 65. The toxicity of ammonium compounds for tobacco. 96

BEAUMONT, A. B., EISENMENGER, W. S., and MOORE, W. J., Jr. (1933). *J. agric. Res.* **47** : 495. Assimilation of fixed nitrogen by grasses and clovers. 95, 97

BEAUMONT, A. B., and LARSINOS, G. J. (1929). *J. Amer. Soc. Agron.* **21** : 150. A water culture technique for studies in tobacco nutrition. 149

BEAUMONT, A. B., LARSINOS, G. J., PIEKENBROCK, P., and NELSON, P. R. (1931). *J. agric. Res.* **43** : 559. The assimilation of nitrogen by tobacco. 97, 149

BEAUMONT, A. B., see THELIN, G.

BECKENBACH, J. R., WADLEIGH, C. H., and SHIVE, J. W. (1936). *Soil Sci.* **41** : 469. A statistical interpretation of the nutrient ion effect upon growth in artificial cultures. 88, 90, 118, 167

BECKENBACH, J. R., see WADLEIGH, C. H.

BECKWITH, F. H. (Private communication 1950). 66

BEESON, K. C. (1941). *Bull. U.S. Dep. Agric.* **369**. The mineral composition of crops with particular reference to the soils in which they were grown. 163

BEGG, R. W., see Young, E. G.

BEHRENS, W. U. (1928) *Z. PflErnähr. Düng.* A **11** : 93. Studien über die Aufnahme und Verwertung der Pflanzennährstoffe. 9, 87, 120, 123, 124

BENNISTER, H. L., and KING, A. (1938). *J. chem. Soc.*, p. 1885. Studies in chemisorption on charcoal. XII. The isoelectric point. 72

BERG, R. O., see Zimmerman, P. W.

BERTRAND, D. (1939). *C.R. Acad. Sci.* (*Paris*) **208** : 2024. Sur la diffusion du molybdène chez les végétaux. 164

BERTRAND, D. (1940). *C.R. Acad. Sci.* (*Paris*) **211** : 512. Importance du molybdène comme oligoélément chez les végétaux. 64, 67

BERTRAND, D. (1941). *C.R. Acad. Sci.* (*Paris*) **213** : 254. L'importance de l'oligoélément vanadium pour l'*Aspergillus niger*. 81

BERTRAND, G. (1912). *Bull. Soc. chim. Fr.* IV. **11** : 401. Sur l'extraordinaire sensibilité de l'*Aspergillus niger* vis à vis du manganèse. 71, 81

BERTRAND, G. (1945a). *Ann. agron. Paris.* **15** : 153. Sur l'origine multiple du magnésium contenu dans l'eau de pluie. 44

BERTRAND, G. (1945b). *Ann. agron. Paris.* **15** : 343. Sur le potassium de l'eau de pluie. 44

BERTRAND, G., and JAVILLIER, M. (1906). *C.R. Acad. Sci.* (*Paris*) **143** : 900. Sur un méthode extrêmement sensible de précipitation du zinc. 73

BERTRAND, G., and JAVILLIER, M. (1911). *C.R. Acad. Sci.* (*Paris*) **152** : 225. Action du manganèse sur le développement de l'*Aspergillus niger*. 3, 66, 82

BERTRAND, G., and JAVILLIER, M. (1912a). *Ann. Inst. Pasteur* **26** : 241. Action combinée du manganèse et du zinc sur le développement et la composition minérale de l'*Aspergillus niger*. 3, 4, 22, 66

BERTRAND, G., and JAVILLIER, M. (1912b). *Bull. Soc. chim. Fr.* IV. **11** : 212. Action du manganèse sur le développement de l'*Aspergillus niger*. 3, 66

BERTRAND, G., and SILBERSTEIN, L. (1941). *C.R. Acad. Sci.* (*Paris*) **213** : 221. Sur la teneur en bor des graines. 164

BETTY, R. L., see JENSEN, H. L.

BIEBEL, J. P., see WITHROW, R. B.

BIEKART, H. M., and CONNORS, C. H. (1935). *Bull. New Jersey agric. Exp. Stat.* **588**. The greenhouse cultivation of carnations in sand. 143

BIRNER, H., and LUCANUS, B. (1866). *Landw. VersSta.* **8** : 128. Wasserculturversuche mit Hafer in der Agrik.-chem. Versuchsstation zu Regenwalde. 11, 14, T19A, 104, 151

BISHOP, W. B. S. (1928). *Aust. J. exp. Biol. med. Sci.* **5** : 125. Distribution of manganese in plants and its importance in plant metabolism. 20, 21, 23, 31, 67

BITCOVER, E. H., and WANDER, I. W. (1950). *Plant Physiol.* **25** : 461. Some observations on nitrate formation and the absorption of nitrogen by *Citrus*. 97

BLACKMAN, G. E., and RUTTER, A. J. (1949). *Ann. Bot. Lond.* **13** n.s. : 453. Physiological and ecological studies in the analysis of plant environment. IV. The interaction between light intensity and mineral nutrient supply on the uptake of nutrients by the bluebell (*Scilla non-scripta*). 159

BLAKE, G. G. (1945a). *J. sci. Instrum.* **22** : 174. A device for indicating small changes in electrolytic resistance. 62

BLAKE, G. G. (1945b). *Chem. Industr.* Jan. 19, p. 28. A new method for rapid comparison and measurement of solution concentration which also provides for the automatic control of solution strength. 127

BLAKE, G. G. (1946). *Chem. Industr.* Feb. 2, p. 46. An electromagnetic liquid flow control value. 127

BLAKE, M. A., NIGHTINGALE, G. T., and DAVIDSON, O. W. (1937). *Bull. New Jersey Agric. Exp. Stat.* **626**. Nutrition of apple trees. 5, 8, 32, T19B, 141, 143

BLAKE, M.A., see DAVIDSON, O. W. ; NIGHTINGALE, G. T. ; TIEDJENS, V. A.

BLANCHARD, F. A., and DILLER, V. M. (1950). *Plant Physiol.* **25** : 767. Technique for growing plants with roots in a sterile medium. 150

BLANK, L. M. (1941). *J. agric. Res.* **62** : 129. Response of *Phomatotrichum omnivorum* to certain trace elements. 74

BLOOD, H. L., see RICHARDS, L. A.

BOLAS, B. D. (1933). *A.R. Cheshunt exp. Res. Stat.* **19** : 84. Influence of light and temperature on assimilation rate of seedling tomato plants. 160

BOLAS, B. D., and PORTSMOUTH, G. B. (1948, unpublished). Report No. **10,536** to the Mineral Deficiencies Conference of the Agricultural Research Council. " Investigations on physiological effects of manganese on the potato plant carried out in the Research Institute of Plant Physiology, Imperial College." 153

BOLAS, B. D., and RUCK, H. C. (1949, unpublished). Report No. **11,657** to the Agricultural Research Council Mineral Deficiencies Conference. Further work in the season 1948/49 on the manganese relations of the potato plant. 17, 153

BOLAS, B. D., see ASHBY, E.

BOLL, W. G., and STREET, H. E. (1951). *New Phytol.* **50** : 52. Studies on the growth of excised roots. 1. The stimulatory effect of molybdenum and copper on the growth of excised tomato roots. 55, 57, 66, 68

BOND, G. (1950). *Ann. Bot. Lond.* **14** n.s. : 245. Symbiosis of leguminous plants and nodule bacteria. 3. Observations on the growth of soya beans in water culture. 133, 165, 166

BOND, G., see VANTSIS, T. U.

BONNER, J., and GREENE, J. (1938). *Bot. Gaz.* **100** : 226. Vitamin B_1 and the growth of green plants. 115

BONNER, J., and GREENE, J. (1939). *Bot. Gaz.* **101** : 491. Further experiments on the relation of vitamin B_1 to the growth of green plants. 115

BORTELS, H. (1927). *Biochem. Z.* **182** : 301. Über die Bedeutung von Eisen, Zink und Kupfer für Mikroorganismen. 72

BORTELS, H. (1930). *Arch. Mikrobiol.* **1** : 333. Molybden als Katalysator bei der biologischen Stickstoffbindung. 4

BORTHWICK, H. A. (1946). *Soil Sci.* **62** : 99. Photoperiodic response as a factor in choice of plants for testing soil deficiencies. 156, 159

BORTHWICK, H. A., see PARKER, M. W.

BORTNER, C. E., and KARRAKER, P. E. (1940). *J. Amer. Soc. Agron.* **32** : 195. Studies of frenching of tobacco with particular reference to thallium toxicity. 155

BOTTOMLEY, W. B. (1914). *Ann. Bot. Lond.* **28** : 531. The significance of certain food substances for plant growth. 115

BOTTOMLEY, W. B. (1915). *Proc. roy. Soc.* (London) **B 88** : 237. Some accessory factors in plant growth and nutrition. 115

BOTTOMLEY, W. B. (1917). *Proc. roy. Soc.* (London) **B 89** : 481. Some effects of organic growth promoting substances (auximones) on the growth of *Lemna minor* in mineral culture solutions. 115

BOTTOMLEY, W. B. (1920a). *Proc. roy. Soc.* (London) **B 91** : 83. The effect of nitrogen fixing organisms and nucleic acid derivatives on plant growth. 115

BOTTOMLEY, W. B. (1920b). *Ann. Bot. Lond.* **34** : 345. The growth of *Lemna* plants in mineral solutions and in their natural medium. 115

BOTTOMLEY, W. B. (1920c). *Ann. Bot. Lond.* **34** : 353. The effect of organic matter on the growth of various water plants in cultural solutions. 115

BOULD, C. (1939). *J. Pomol.* **17** : 254. Studies on the nutrition of tulips and *Narcissi*. 18

BOUSSINGAULT, J. B. (1886). (Not seen). " Agronomie chimie agricole et physiologie " 3rd Edition. Paris. Recherches sur la végétation. 2, 26

BOWER, C. A., and WADLEIGH, C. H. (1948). *Proc. Soil Sci. Soc. Amer.* **13** : 218. Growth and cationic accumulation by four species of plants as influenced by various levels of exchangeable sodium. 36

BOYCE THOMPSON INSTITUTE (Anon) (1925). *Contr. Boyce Thompson Inst.* **1** : 3. Organisation, equipment and dedication. 162

BRANDENBURG, E. (1933). *Tijdschr. PlZiekt.* **39** : 189. Onderzoekingen over ontginningsziekte II. 17, 22, 54, 70, 105, 113

BRANDENBURG, E. (1935). *Meded. Inst. Suckerbiet Bergen op. Z.* **5** : 245. Ontginningsziekte en kopergebrek. 70, 109, 151

BRANDON, D. (1946). " Artificial illumination in Horticulture." Tech. Rep. W/T **11**. British Electrical and Allied Traders' Research Association, London. 163

BREAZEALE, J. F. (1905). *Science* **22** : 146. Effect of the concentration of the nutrient solution upon wheat cultures. 155

BREAZEALE, J. F. (1923). *J. agric. Res.* **24** : 41. Nutrition of plants considered as electrical phenomena. 11, 14, 139

BREAZEALE, J. F. (1928). *Tech. Bull. Ariz. agric. Exp. Stat.* **21**. Soil zeolites and plant growth. 35

BREAZEALE, J. F., see MCGEORGE, W. T.

BRENCHLEY, W. E. (Private communications). 10, 15, 16, 17, 22, 23, 52, 67, 68, 85, T19A, 100, 104, 108, 117, 135, 151, 158

BRENCHLEY, W. E. (1910). *Ann. Bot. Lond.* **24** : 571. The influence of copper and manganese sulphate upon the growth of barley. 51, 83, 112, 113, 169

BRENCHLEY, W. E. (1914). *Ann. Bot. Lond.* **28** : 283. On the action of certain compounds of zinc, arsenic and boron on the growth of plants. 51, 52, 83, 112, 113, 165, 169

BRENCHLEY, W. E. (1916). *Ann. Bot. Lond.* **30** : 77. The effect of the concentration of the nutrient solution on the growth of barley and wheat in water cultures. 52, 88, 161, 165

BRENCHLEY, W. E. (1923). *Ann. appl. Biol.* **10** : 223. Effect of weight of seed on the resulting crops. 165

BRENCHLEY, W. E. (1929). *Ann. Bot. London.* **43** : 89. The phosphate requirements of barley at different periods of growth. 86, 139

BRENCHLEY, W. E. (1946). *Chemical Products*, May-June. The rôle of minor elements in the growth of plants. 64, 65

BRENCHLEY, W. E., and KHARAK-SINGH (1922). *Ann. appl. Biol.* **9** : 197. Effect of high root temperature and excessive insolation upon growth. 160

BRENCHLEY, W. E., and WARINGTON, K. (1927). *Ann. Bot. Lond.* **41** : 167. The rôle of boron in the growth of plants. 67, 96, 114

BRENCHLEY, W. E., and WARINGTON, K. (1945 unpublished). Report No. **7874**. to the Mineral Deficiencies Conference of the Agricultural Research Council. "Report on flax experiments. 1944." 104

BRENCHLEY, W. E., see GREGORY, F. G.; HALL, A. D.

BREWER, K. A. (1941). *J. chem. Educ.* **18** : 217. Isotopes in the study of plant growth. 117

BRIGGS, G. E., KIDD, F., and WEST, C. (1920). *Ann. appl. Biol.* **7** : 202. A quantitative study of plant growth. 168

BRIGGS, G. E., see WEST, C.

BRIGGS, L. J., and SHANTZ, H. L. (1911). *Bot. Gaz.* **51** : 210. A wax seal method for determining the lower limit of available soil moisture. 137

BRIGGS, L. J., and SHANTZ, H. L. (1912). *Bull. U.S. Dep. Agric. Bur. Plant Industry.* **230**. The wilting coefficient for different plants and its indirect determination. 137

VON BRONSART, H. (1949). *Z. PflErnähr. Düng.* 89 (n.s. **44**) : 119. Lösliche Spurenelemente im Hohenbockaer Glassand. 29

BROWN, D. S. (1945). *Bull. W. Va agric. Exp. Stat.* **322**. The growth and composition of the tops of peach trees in sand culture in relation to nutrient balance. 8, 18, 47, 48, 118, 141

BROWN, E. M. (1939). *Plant Physiol.* **14** : 517. Equipment for growing plants at controlled temperatures. 161

BROWN, S. M., see CHAPMAN, H. D.

BROYER, T. C., and FURNSTALL, A. H. (1945). *Plant Physiol.* **20** : 690. A note on the effects of copper impurities in distilled water on growth of plants. 51

BROYER, T. C., see BARKER, H. A.; HOAGLAND, D. R.

BRUCH, F. (1902). *Landw. Jahrb.* **30** (3) : 127. Zur physiologischen Bedeutung des Kalziums in der Pflanze. T19A, 105, 106, 107

BUCKNER, G. D. (1915). *J. agric. Res.* **5** : 449. The translocation of mineral constituents of seeds and tubers of certain plants during growth. 164

BURK, D., see HORNER, C. K.

BURKHART, L., see LINEBERRY, R. A.

BURKHOLDER, P. R., and MCVEIGH, I (1940). *Amer. J. Bot.* **27** : 414. Growth and differentiation of maize in relation to nitrogen. 165

BURRIS, R. H., see GOSH, B. P.

BURSTRÖM, H. (1945). *Ann. agric. Coll. Sweden* **13** : 1. The nitrate nutrition of plants. 159

BURSTRÖM, H., see GELIN, O. E. V.

BUSHELL, R. J., see WALLACE, R. H.

CALFEE, R. K., see MCHARGUE, J. S.

CAMPBELL, W. A., and PRESLEY, J. T. (1945). *Phytopathology*, **35** : 213. Design for constant temperature tanks. 161

CARRERO, J. O., see GILE, P. L.

CARROLL, G. H. (1939). *Plant Physiol.* **14** : 603. Sintered pyrex glass aerators. 137

CARTER, W. (1942). *Phytopathology*, **32** : 623. A method of growing plants in water vapour to facilitate examination of roots. 122

CHANDLER, F. B. (1941). *Bull. Me agric. Exp. Stat.* **404**. Mineral nutrition of the genus *Brassica* with particular reference to boron. 21, 22, 30, 31, 84, T19B, 120, 155, 165

CHANDLER, F. B. (1944). *Soil Sci.* **57** : 67. Nutrition of *Brassica* and potatoes. 120

CHANDLER, W. H., see HOAGLAND, D. R.

CHANG, H. T., and LOOMIS, W. E. (1945). *Plant Physiol.* **20** : 221. Effect of carbon dioxide on absorption of water and nutrients by roots. 133

CHAPMAN, H. D. (Private communications). 129, 141, 143, 144

CHAPMAN, H. D. (1939). *Soil Sci.* **48** : 309. Absorption of iron from finely ground magnetite. 30, 41, 102, 103

CHAPMAN, H. D., and BROWN, S. M. (1943). *Soil Sci.* **55** : 87. Potash in relation to citrus nutrition. 143, 152

CHAPMAN, H. D., BROWN, S. M., and LIEBIG, G. F. Jr. (1943). *California Citrograph* **28** : 198, 211, 230, 246. Some effects on *Citrus* fruit quality of nitrogen, phosphorus and potassium. 143

CHAPMAN, H. D., BROWN, S. M., and RAYNER, D. S. (1947). *Hilgardia* **17** : 619. Effects of potash deficiency and excess on orange trees. 13, 87, 143, 160, 161

CHAPMAN, H. D., and LIEBIG, G. F. Jr. (1937). *Proc. Soil Sci. Soc. Amer.* **2** : 359. The effects of various nitrate concentrations on the growth and composition of sweet orange seedlings. 129, 143

CHAPMAN, H. D., and LIEBIG, G. F., Jr. (1938). *J. agric. Res.* **56** : 73. Adaptation and operation of automatically operated sand culture equipment. 5, 9, 21, 102, 103, 118, 129, 132, 135, 143, 147

CHAPMAN, H. D., and LIEBIG, G. F., Jr. (1940). *Hilgardia* **13** : 141. Nitrate concentration and ion balance in relation to citrus culture. 6, 9, 87, 102, 117, 119, 133, 143, 155

CHAPMAN, H. D., LIEBIG, G. F., Jr., and VANSELOW, A. P. (1939). *Proc. Soil Sci. Soc. Amer.* **4** : 196. Some nutritional interrelationships as revealed by a study of mineral deficiency and excess symptoms in *Citrus*. 46, 143, 147

CHAPMAN, H. D., and PARKER, E. R. (1942). *Plant Physiol.* **17** : 366. Weekly absorption of nitrate by young bearing orange trees growing out of doors in solution cultures. 13, T19B, 135, 143

CHAPMAN, H. D., VANSELOW, A. P., and LIEBIG, G. F., Jr. (1937). *J. agric. Res.* **55** : 365. The production of citrus mottle leaf in controlled nutrient cultures. 15, 16, 17, 22, 54, 66, 67, 68, 82, T19B, 109, 114, 115, 143, 152, 159

CHAPMAN, H. D., see GUEST, P. L. ; LIEBIG, G. F., Jr.

CHESTERS, C. G. P., and STREET, H. E. (1948). *Ann. appl. Bot.* **35** : 443. Studies in plant nutrition. 1. The effect of some organic supplements on the growth of lettuce in sand culture. 110, 111, 115, 116

CHILDERS, N. F., see CIBES, H. R. ; WHITE, D. G.

CHOUCHAK, D., see POUGET, I.

CIBES, H. R., CHILDERS, N. R., and LOUSTALOT, A. J. (1947). *Plant Physiol.* **22** : 291. Influence of mineral deficiencies on growth and composition of vanilla leaves. 26, 29

CLARK, H. E., and SHIVE, J. W. (1932). *Soil Sci.* **34** : 37. Influence of continuous aeration upon the growth of tomato plants in solution cultures. T19B, 135

CLARK, H. E., and SHIVE, J. W. (1934). *Soil Sci.* **37** : 203. The influence of the pH of a culture solution on the rates of absorption of ammonium and nitrate nitrogen by the tomato plant. 13, 93, 95, 100, 101, 119

CLARK, J. H. (1933). *Proc. Amer. Soc. hort. Sci.* **30** : 283. Reaction of the nutrient medium as affecting the growth of strawberry plants. 9, 21, 93, 100, 119

CLARK, J. H. (1941). *Bull. New Jersey agric. Exp. Stat.* **691**. Growth and composition of the strawberry plant as affected by source of nitrogen and pH value of the nutrient medium. 9, 19, 94, 95, 100, 101, 118, 119

CLARK, N. A. (1926). *Plant Physiol.* **1** : 273. Plant growth promoting substances, hydrogen ion concentration and the reproduction of *Lemna*. 115

CLARK, O. L. (1916). *Science* **44** : 868. A method for maintaining a constant volume of nutrient solutions. 120, 122

CLEMENTS, H. F. (1928). *Plant Physiol.* **3** : 441. Plant nutrition studies in relation to the triangular system of water cultures. 12, 160, 165, 166, 167

CLIQUET, R., GUILBERT, J., and PENAN, H. (1933). *J. Pharm. Chim Paris* **18** : 321. Preparation industrielle d'eau bi-distillée pour usage biologique. Charactéristiques physico-chimiques de l'eau. 54, 55

COCHRAN, W. G., see COX, G. M.

COLBY, H. L. (1933). *Plant Physiol.* **8** : 1. Seasonal absorption of nutrient salts by the French prune grown in solution cultures. 12, 16, 21, T19B, 108, 143

COLLISON, R. C., and MENSCHING, J. E. (1932). *Bull. New York State agric. Exp. Stat.* **193**. Lysimeter investigations. II. Composition of rain water at Geneva N.Y. for a 10-year period. 43

COLWELL, W. E. (1943). *Soil Sci.* **56** : 71. A biological method for determining the relative boron contents of soils. 6, 7, 18, 29, T7, T19B, 159, 160, 165

COLWELL, W. E., and CUMMINGS, R. W. (1944). *Soil Sci.* **57** : 37. Chemical and biological studies on aqueous solutions of boric acid and of calcium, sodium and potassium metaborates. 114

COLWELL, W. E., see MEHLICH, A.

CONNER, S. D. (1920). *J. Amer. Soc. Agron.* **12** : 61. The effect of zinc in soil tests with zinc and galvanised iron pots. 18

CONNORS, C. H., see BIEKHART, H. M.

CONVERSE, C. D., GAMMON, N., and SAYRE, J. D. (1943). *Plant Physiol.* **18** : 114. Use of ion exchange materials in studies on maize nutrition. 35, 36

COOK, R. L., and MILLAR, C. E. (1946). *Proc. Soil Sci. Soc. Amer.* **11** : 298. Some techniques which help to make greenhouse investigations comparable with field crop experiments. 6, 166, 170

COSTA, A. S., see FRANCO, C. M.

COWAN, E. W., see JENNY, H.

COX, G. M., and COCHRAN, W. G. (1946). *Soil Sci.* **62** : 87. Designs of greenhouse experiments for statistical analysis. 166, 167

Crist, J. W., see Morris, S.

Crocker, W. (Editor) *et al.* (1948). "Growth of Plants" (Twenty years' research at Boyce Thompson Institute). Reinhold, New York. 152, 161, 162

Crone, G. van der (1904) (not seen). Ergebnisse von Untersuchungen über die Wirkung der Phosphorsäure auf die höheren Pflanzen und eine neue Nahrlösung. Dissertation Univ. Bonn. 3, 84, T19A, 104, 106, 107, 108, 156

Crowther, C., and Rustan, H. G. (1911). *J. agric. Sci.* **4** : 25. The nature, distribution and effects upon vegetation of atmospheric impurities in and near an industrial town. 42, 43

Crowther, C., and Steuart, D. W. (1912). *J. agric. Sci.* **5** : 390. The distribution of atmospheric impurities in the neighbourhood of an industrial city. 42

Crowther, F., see Gregory, F. G.

Cullinan, F. P., and Batjer, L. P. (1943). *Soil Sci.* **55** : 49. Nitrogen, phosphorus and potassium interrelationships in young peach and apple trees. 87, 88, 141

Cullinan, F. P., Scott, D. H., and Waugh, J. G. (1938). *Proc. Amer. Soc. hort. Sci.* **36** : 61. The effects of varying amounts of nitrogen, potassium and phosphorus on the growth of young peach trees. 8, 21, 118, 119, 141, 143

Cullinan, F. P., see Weinberger, J. H.

Cummings, R. W., see Colwell, W. E.

Curtin, L. P., and Parker, H. C. (1940). *J. Soc. Glass. Tech.* **24** (Abs) : 198. The purification of sand. 31, 175

Curtis, D. S. (1949a). *Soil Sci.* **67** : 253. Effect of oxygen supply in nutrient solution on avocado and citrus seedlings. 57, T19B, 133

Curtis, D. S. (1949b). *Soil Sci.* **68** : 441. Nitrite injury on avocado and citrus seedlings in nutrient solution. 97, 133

Cutts, H. E. (1935). *Industr. Engng. Chem.* **27** : 1491. Composition of nitrogenous fertiliser salts sold in the American market. 64, 65

Datta, N. P., see Vanselow, A. P.

Davidson, O. W. (1928). *Proc. Amer. Soc. hort. Sci.* **25** : 354. An application of the triangle system in determining a nutrient solution suitable for research with the peach tree in sand culture. 9, 96

Davidson, O. W. (1944). *Soil Sci.* **58** : 51. The translocation of potassium among peach roots. 15, 26, T19B

Davidson, O. W. (1946). *Soil Sci.* **62** : 71. Large scale soilless culture for plant research. 6, 18, 19, T4, 27, 48, 143, 144, 145, 147

Davidson, O. W., and Blake, M. A. (1937). *Proc. Amer. Soc. hort. Sci.* **35** : 339. Nutrient deficiency and nutrient balance with the peach. 87, 141

Davidson, O. W., and Shive, J. W. (1934). *Soil Sci.* **37** : 357. The influence of hydrogen ion concentration of the culture solution upon the absorption and assimilation of nitrate and ammonium nitrogen by peach trees grown in sand cultures. 5, 9, 21, 93, 95, T19B, 100, 103

Davidson, O. W., see Blake, M. A.

Davies, E. B. (1949, Private communication). 19, 151

Davies, G. N. (1941). *Ann. appl. Biol.* **28** : 81. An investigation of the effect of zinc sulphate on plants. 169

Davis, A. R. (1921). *Soil Sci.* **11** : 1. The variability of plants grown in water culture. 165, 166

Davis, A. R., and Hoagland, D. R. (1928). *Plant Physiol.* **3** : 277. An apparatus for the growth of plants in a controlled environment. 152, 159, 161, 165

Davis, A. R., see Vlamis, J.

Davis, J. F., see Schroeder, W. T.

Davis, M. B. (1927). *Sci. Agric.* **8** : 41. Influence of ammonium sulphate as a direct source of nitrogen for apple trees. T7, 98, 149

Davis, M. B. (1930). *J. Pomol.* **8** : 316. Investigations on the nutrition of fruit trees. Some effects of deficiencies of nitrogen, potassium, calcium and magnesium with special reference to the behaviour of certain varieties of apple trees. 5, 8, 19, 48, T19B, 141

Davis, M. B., Hill, H., and Johnson, F. B. (1934). *Sci. Agric.* **14** : 411. Nutritional studies with *Fragaria*. 2. A study of the effect of deficient and excess potassium, phosphorus, magnesium, calcium and sulphur. 7, 19, 118

Dean, L. A., and Rubens, E. J. (1945). *Soil Sci.* **59** : 437. Absorption by plants of phosphorus from a clay-water system. Methods and ensuing observations. 39

Degman, E. S., see Batjer, L. P.

Demolon, A., and Bastisse, E. (1941). *C.R. Acad. Sci. (Paris)* **212** : 1002. Liberation of sulphur and sulphur balance in soil. 43

Demoussy, E., see Maquenne, L.

Detmer, W. (1898). "Practical Plant Physiology." Translated by S. A. Moore. (London). T19A, 106, 107

De Turk, E. E., see Hoener, I. R.

Deuber, G. G. (1926). *Soil Sci.* **21** : 23. Potassium ferrocyanide and ferric ferrocyanide as sources of iron for plants. 105

Dikussar, I., see Mevius, W.

Diller, V. M., see Blanchard, F. A.

Dobson, G. M. B. (Chairman) (1945). "Atmospheric pollution in Leicester." A scientific survey. *Tech. Pap. D.S.I.R.* Atmospheric Pollution Research No. 1. 42, 43

Domontovitch, M. K., see Prianishnikov, D. N.

Dorph-Petersen, K., and Steenbjerg, F. (1950). *Plant and Soil* **2** : 283. Investigations of the effect of fertilizers containing sodium. 92, 93

Drosdoff, M., see Loustalot, A. J.

DuBuy, H. G. (1939). *Plant Physiol.* **14** : 832. Metering pump and turntable arrangement for supplying measured quantities of nutrient solution successively to any number of culture vessels. 127

Duckert, R., see Wenger, P.

Duggar, B. M. (1920a). *Ann. Mo. bot. Gdn.* **7** : 1. Hydrogen ion concentration and the composition of nutrient solutions in relation to the growth of seed plants. 104, 105, 108

Duggar, B. M. (1920b). *Ann. Mo. bot. Gdn.* **7** : 307. The use of insoluble salts in balanced solutions for seed plants. 10, 14, 104

Duhamel du Monceau, H. L. (1758). "La Physique des Arbres." (Paris) **2** : 202. 2, 42, 141

Duncan, D. R. (1935). "The Painting of Galvanised Iron." *Tech. pap.* No. **62**. *Res. Ass. Brit. Paint, Colour and Varnish Manufrs.* 47

Dunn, S. (1939). *Plant Physiol.* **14** : 836. Convenient seedling support for growing plants in water culture. 16

Durell, W. D. (1941). *Plant Physiol.* **16** : 327. The effect of aeration on the growth of the tomato in nutrient solution. 133

Dustman, R. B. (1925). *Bot. Gaz.* **79** : 233. Inherent factors related to absorption of mineral elements by plants. 137

Early, E. B. (1943). *J. Amer. Soc. Agron.* **35** : 1012. Minor element studies with soya beans. 1. Varietal reactions to concentrations of zinc in excess of the nutritional requirements. 46, 113, 147

Eastes, J. W., see Myers, R. J.

Eaton, F. M. (1931). *Soil Sci.* **31** : 235. Large-scale sand culture apparatus. 5, 9, 18, T4, T19B, 119, 144, 147

Eaton, F. M. (1936). *J. agric. Res.* **53** : 433. Automatically operated sand culture equipment. 5, 9, 14, 16, 18, 21, T4, 26, 27, 28, 30, 48, 92, 96, T19B, 102, 103, 117, 129, 144, 146, 147, 148, 164

Eaton, F. M. (1941a). *Plant Physiol.* **16** : 385. Plant culture equipment. 5, 9, 12, 18, 19, 21, T4, 26, 102, 129, 131, 134, 135, 137, 144, 147, 166

Eaton, F. M. (1941b). *Plant Physiol.* **16** : 545. Water uptake and root growth as influenced by irregularities in the concentration of the substrate. 25, 91, 113

Eaton, F. M. (1941c). *Plant Physiol.* **16** : 834. Use of nitric acid in control of pH and nitrate levels in nutrient solutions. 50, 99

Eaton, F. M. (1942a). *J. agric. Res.* **64** : 357. Toxicity and accumulation of chloride and sulphate salts in plants. 9, 48, 50, 91, 145

Eaton, F. M. (1942b). *Chron. Bot.* **7** : 200. Sand culture methods. 118, 144, 145

Eaton, F. M. (1944). *J. agric. Res.* **69** : 237. Deficiency, toxicity and accumulation of boron in plants. 9, 48, 49, 50, 112, 145, 159

Edelman, C. H. (1939). (Not seen ; cited by Lehr 1940, q.v.). 43

Edgerton, L. J. (1942). *Proc. Amer. Soc. hort. Sci.* **41** : 237. The use of metaphosphate in nutrient solutions. 99, 106

Edmonson, Y. H., see Thiman, K. V.

Ehrenberg, P. (1910). *Landw. VersSta.* **72** : 15. Wirkungen des Zinks bei Vegetationsversuchen. 18

Eisenmenger, W. S., see Beaumont, A. B.

Eltinge, E. T. (1936). *Plant Physiol.* **11** : 767. Effect of boron deficiency upon the structure of *Zea mays*. 51, 67, 155

Emmerie, A., see Wolfe, L. K.

Erdman, L. W. (1922). *Soil Sci.* **14** : 363. The sulphur content of rainwater. 43

Erdman, L. W., see Bakke, A. L.

Erickson, L. C. (1946). *Amer. J. Bot.* **33** : 551. Growth of tomato roots as influenced by oxygen in the nutrient solution. 133

Erkama, J. (1947). *Ann. Acad. Sci. Fenn.* Ser. A. II (Chim). **25** : 1. Über die Rolle von Kupfer und Mangan im Leben der höheren Pflanzen. 164

Evans, H. (1950). *Cocoa Conference Proc.* (London), 1950, p. 20. Report on cocoa investigations in progress in Trinidad with a summary of results achieved to date. 4, 27, 133, 143, 153

Evans, H. (1951, private communications). 5, 27, 48, 124, 133, 156

Evans, H., and Maskell, E. J. (1950, private communications). 128, 135

Evans, H. J., Purvis, E. R., and Bear, F. E. (1950). *Plant Physiol.* **25** : 553. Molybdenum nutrition of alfalfa. 15, 17, 22, 26, 51, 66, 71, 79, 82

Evans, H. J., Purvis, E. R., and Bear, F. E. (1951). *Soil Sci.* **71** : 117. Effect of soil reaction on availability of molybdenum. 66

Evans, U. R. (1946). "The corrosion of metals." 2nd Ed. London. 46, 47

Fales, H. A. (1928). "Inorganic quantitative analysis." Bell & Co., London. 81

Farnham, R. B., see Nightingale, G. T.

Feigl, F. (1949). "Specific selective acid sensitive reactions." Translated by R. E. Oesper. Academic Press New York. 80

Fenstel, I. C., see Gile, P. L.

Ferguson, W., and Wright, E. L. (1940). *Sci. Agric.* **20** : 8. Micro element studies with special reference to the element boron. 41, 111

Ferres, H. M., and Trumble, H. C. (1943). *J. Aust. Inst. agric. Sci.* **9** : 179. Exploratory investigations of soil deficiencies by means of small pot cultures. 6

Ferres, H. M., see Trumble, H. C.

Fielding, A. H., see Nicholas, D. J. D.

Fisher, R. A. (1942). "The design of experiments." Oliver & Boyd, Edinburgh. 167

FITTING, H. (1928). *Jb. wiss. Bot.* **67** : 427. Untersuchungen über Chemokinese bei *Vallisneria*. 52, 54, 55

FLEMION, F. (1938). *Contr. Boyce Thomp. Inst.* **9** : 339. A rapid method for determining the viability of dormant seeds. 154

FLEMION, F. (1941). *Contr. Boyce Thomp. Inst.* **11** : 455. Further studies on rapid determination of the germinative capacity of seeds. 154

FOGG, G. E. (1948). *Ann. Bot. Lond.* **12** n.s. : 457. An apparatus for the culture of algae at constant temperature. 161

FORBES, G. S., see LEIGHTON, W. G.

FRANCO, C. M., and COSTA, A. S. (1949). *Plant Physiol.* **24** : 320. A simple aerator for nutrient solutions. 135

FRANCO, C. M., and LOOMIS, W. E. (1947). *Plant Physiol.* **22** : 627. The absorption of phosphorus and iron from nutrient solutions. 10

FRANK (1893). Given by Anderson (1944a). 24

FRANKLIN. Cited by MILLER, N. M. J. (1905). 43

FRAPS, G. S., and STURGES, A. J. (1935). *Bull. Tex. agric. Exp. Stat.* **515**. Availability of nitrous nitrogen to plants. 97

FRATSKE, W. E., see ARNON, D. I.

FRED, E. B., see HOPKINS, E. W.

FRENCH, R. B. (1937). *A.R. Fla agric. Exp. Stat.* p. 68. Nutrient concentration in the soil with special reference to the trace elements. 106, 107, 108

FURNSTALL, A. H., and JOHNSTON, B. (1936). *Plant Physiol.* **11** : 189. Preparation of sintered pyrex glass aerators for use in water culture experiments with plants. 137

FURNSTALL, A. H., see ARNON, D. I. ; BROYER, T. C.

FURR, J. R., REECE, P. C., and GARDNER, F. E. (1946). *Proc. Fla St. hort. Soc.* pp. 138. Symptoms exhibited by avocado trees grown in outdoor sand cultures deprived of various mineral nutrients. 153

GADD, C. H. (1928). *Tea Quart.*, **1** : 31. Tea and soil acidity. Water culture experiments I and II. 99, 143

GADDUM, L. W., and ROGERS, L. H. (1936). *Bull. Fla agric. Exp. Stat.* **290**. A study of some trace elements in fertiliser materials. 64, 65

GALE, C. F. (1949). *J. gen. Microbiol.* **3** : 369. The assimilation of amino acids by bacteria. VIII. Trace metals in glutamic acid assimilation and their inactivation by 8-hydroxyquinoline. 81

GALHAC, E., see JAULMES, P.

GALLEGLY, M. E., and WALKER, J. C. (1949). *Amer. J. Bot.* **36** : 613. Plant nutrition in relation to disease. V. Bacterial wilt of tomato. 120

GARDNER, F. E., see ROY, W. R.

GAUCH, H. G. (Private communication). 131

GAUCH, H. G., and MAGISTAD, O. C. (1943). *J. Amer. Soc. Agron.* **35** : 871. Growth of strawberry clover varieties and of alfalfa and Ladino clover as affected by salt. 91, T19B, 108, 151

GAUCH, H. G., and WADLEIGH, C. H. (1943). *Plant Physiol.* **18** : 543. A new type of intermittently-irrigated sand culture equipment. 5, 9, 21, T4, 27, 28, 131, 136

GAUCH, H. G., and WADLEIGH, C. H. (1945). *Soil Science* **59** : 139. Effect of high concentrations of sodium, calcium, chloride and sulphate on ionic absorption by bean plants. 50, 91, T19B, 99

GAUCH, H. G., see MAGISTAD, O. C.

GELIN, O. E. V., and BURSTRÖM, H. (1949). *Physiol. Plantarum* **2** : 70. A study of artificial illumination of greenhouse cultures. 163

GERICKE, W. F. 105

GERICKE, W. F. (1921). *Amer. J. Bot.* **8** : 59. Influence of temperature on the relation between nutrient salt proportions and the early growth of wheat. 160

GERICKE, W. F. (1925a). *Bot. Gaz.* **79** : 106. Effect of light intensity on severity of Fe chlorosis. 159

GERICKE, W. F. (1925b). *Bot. Gaz.* **80** : 410. Salt requirements of wheat at different growth phases. 86

GERICKE, W. F. (1930). *Soil Sci.* **29** : 207. Plant food requirements of rice. 160

GERICKE, W. F., and TAVERNETTI, J. R. (1936). *Agric. Engng. St. Joseph. Mich.* **17** : 141 and 184. Heating of liquid culture media for tomato production. 144, 160

GERMAR, B. VON (1934). *Z. PflErnähr. Düng.* A**35** : 102. Über einige Wirkungen der Kieselsäure in Getreidepflanzen, insbesondere auf deren Resistenz gegenüber Mehltau. 40

GERSHBERG, S., see HIBBARD, R. P.

GHOSH, B. P., and BURRIS, R. H. (1950). *Soil Sci.* **70** : 187. Utilisation of nitrogenous compounds by plants. 98, 149, 150

GILBERT, B. E., and PEMBER, F. R. (1935). *Soil Sci.* **39** : 425. Tolerance of certain weeds and grasses to toxic aluminium. 119

GILBERT, B. E., see MCLEAN, F. T.

GILBERT, J. H., see LAWES, J. B.

GILBERT, S. G., and SHIVE, J. W. (1942). *Soil Sci.* **53** : 143. Significance of oxygen in nutrient substrates for plants. 1. Oxygen requirement. 133, 137, 149, 159

GILBERT, S. G., and SHIVE, J. W. (1945). *Soil Sci.* **59** : 453. The importance of oxygen in the nutrient substrate. Relation of the nitrate ion to respiration. 133

GILBERT, S. G., see LOUSTALOT, A. J.

GILE, P. L. (1938). *J. agric. Res.* **56** : 787. A measure of toxicity in plant studies. 28, 169

GILE, P. L. (1939). *J. agric. Res.* **59** : 619. Growth of millet in quartz sand and in sand-soil mixtures. 40

GILE, P. L., and CARRERO, J. O. (1916). *J. agric. Res.* **7** : 503. Assimilation of iron by rice from certain nutrient solutions. T19B, 105, 106

GILE, P. L., and CARRERO, J. O. (1917). *J. agric. Res.* **9** : 73. Absorption of nutrients as affected by the number of roots supplied with the nutrient. 25

GILE, P. L., and CARRERO, J. O. (1921). *J. agric. Res.* **21** : 545. Assimilation of nitrogen, phosphorus and potassium by corn when nutrient salts are confined to different roots. 25

GILE, P. L., and FENSTEL, I. C. (1943). *J. agric. Res.* **66** : 49. Effect of soil and peat admixtures on the growth of plants in quartz sand. T4, 27, 28, 40, 101, 105

GINES, F. G. (1930). *Philipp. Agric.* **19** : 43. Relative effects of different iron salts upon growth and development of young rice plants. 104, 105

GINTER, W. D., see PUCHER, G. W.

GLASSTONE, V. F. C. (1947). *Amer. J. Bot.* **34** : 218. Inorganic micronutrients in tomato root tissue culture. 68, 70

GLEDHILL, W. S., see ALBERT, A.

GOLDSCHMIDT, V. M. (1936). *Industr. Engng. Chem.* **27** : 1100. Rare elements in coal ashes. 43

GOLDSCHMIDT, V. M. (1937). *J. chem. Soc. Lond.* p. 655. The principles of distribution of chemical elements in minerals and rocks. 69

GOLDSCHMIDT, V. M. (1943). Lecture to British Coal Utilisation Research Association, May 8th, 1943. The occurrence of rare elements in coal ashes. 18, 43, 47

GOLLMICK, F. (1935). *Zbl. Bakt.* **93** : 421. Der Einfluss von Zink, Eisen, Kupfer und deren Kombination auf das Wachstum von *Aspergillus niger*. 23, 52, 54, 67, 73

GOODALL, D. W., and GREGORY, F. G. (1947). "Chemical composition of plants as an index of their nutritional status." *Tech. Commun. Bur. Hort. E. Malling* **17**. 141

GOSS, R. W., see PELTIER, G. L.

GOTSCHALL, R., see SCHARRER, K.

GRAHAM, E. R. (1941). *Soil Sci.* **51** : 65. Calcium transfer from mineral to plant by colloidal clay. 38, 39

GRAHAM, E. R., and ALBRECHT, W. A. (1943). *Amer. J. Bot.* **30** : 195. Nitrate absorption by plants as an exchange phenomenon. 36, 38

GRANT, E. P., see HILL, H.

GRAY, A. N. (1944). "Phosphates and superphosphates." 2nd Ed. Keron & Co. London. 64

GREENE, J., see BONNER, J.

GREENHILL, A. W., and PAGE, H. J. (1933). *J. agric. Sci.* **23** : 329. A note on the employment of a mixture of sand and calcium bentonite as the growth medium in pot culture and the establishment therein of a sward of perennial rye grass. 28, 39

GREENWOOD, M. (1951, private communications). 10, 17

GREENWOOD, M., and POSNETTE, A. F. (1947). *Nature* **159** : 542. A morphological change induced in leaves of *Theobroma cacao* L. by mineral deficiency. 143

GREGORY, F. G. 156

GREGORY, F. G. (1917). *Third A.R. Cheshunt exp. Res. Stat.* p. 19. Physiological conditions in cucumber houses. 168

GREGORY, F. G. (1921). *Ann. Bot. Lond.* **35** : 93. Energy relations in plants. 1. The increase in area of leaves and leaf surface of *Cucumis sativus*. 161

GREGORY, F. G. (1928a). *Ann. Bot. Lond.* **42** : 469. Studies in the energy relations of plants. II. The effects of temperature on increase in area of leaf surface and in dry weight of *Cucumis* leaf. 161

GREGORY, F. G. (1928b). *Proc. roy. Soc.* (London) B. **102** : 311. The differential effect of the ions of three salt solutions on the growth of potato plants in sand culture. 90, 167

GREGORY, F. G. (1937). *Ann. Rev. Biochem.* **6** : 557. Mineral nutrition of plants. 87, 93

GREGORY, F. G. (1943). Report No. **7221** to the Mineral Deficiencies Conference of the Agricultural Research Council. "Report on experiments on minor element deficiencies in flax in water culture carried out in 1943." T19B, 105, 110, 152, 159

GREGORY, F. G., and BRENCHLEY, W. E. (1945). Report No. **7861** to the Mineral Deficiencies Conference of the Agricultural Research Council. "Report on work on iron chlorosis in flax." (Carried out in collaboration with Rothamsted Experimental Station 1944). 104, 108, 159, 160

GREGORY, F. G., and CROWTHER, F. (1928). *Ann. Bot. Lond.* **42** : 757. A physiological study of varietal differences in plants. 1. A study of the comparative yields of barley varieties with different manurings. 7, 21, 40, 152

GREGORY, F. G., and RICHARDS, F. J. (1929). *Ann. Bot. Lond.* **43** : 119. Physiological studies in plant nutrition 1. The effect of manurial deficiency on the respiration and assimilation rate in barley. 7, 21, 40, 168

GREGORY, F. G., and SEN, P. K. (1937). *Ann. Bot. Lond.* **1** n.s. : 521. Physiological studies in plant nutrition VI. The relation of respiration rate to the carbohydrate and nitrogen metabolism of the barley leaf as determined by nitrogen and potassium deficiency. 87

GREGORY, F. G., and WOODFORD, E. K. (1939). *Ann. Bot. Lond.* **3** n.s. : 147. An apparatus for the study of the oxygen salt and water uptake of various zones of the root, with some preliminary results with *Vicia Faba*. 137

GREGORY, F. G., see GOODALL, D. W.; HEATH, O. V. S.; WOODFORD, E. K.

GRIS, E. (1844). *C.R. Acad. Sci.* (*Paris*) **19** : 1118. Nouvelles expériences sur l'action de composés ferrugineux solubles appliqué à la végétation et spécialement en traitement de la chlorose et de la débilité des plantes. 2

GROSSENBACHER, K. A., and LIVINGSTONE, B. E. (1936). *Amer. J. Bot.* **23** : 588. Cryptographic malnutrition of sorghum in solution culture. 109, 156

GROSSENBACHER, K. A., see ARNON, D. I.

GUEST, P. L. (1944). *Proc. Amer. Soc. hort. Sci.* **44** : 43. Root contact phenomena in relation to iron nutrition and growth of citrus. 41, 102, 103, 143

GUEST, P. L., and CHAPMAN, H. D. (1944). *Soil Sci.* **58** : 455. Some effects of pH on the growth of citrus in sand and solution cultures. 9, 12, 21, 29, 30, 39, 41, 51, T19B, 98, 99, 101, 102, 103, 107, 113, 114, 143

GUILBERT, J., see CLIQUET, R.

HAAG, J. R., see MCCALL, A. G.

DE HAAN, I. (1941). *Arch. Theecult.* **15** : 1. Gebrekverschijnselen bij thee veroorzaakt door een tekort aan de belangrijkste minerale voedingsstoffen met uitzondering van kalium. 5, 7, 107, 143, 152

DE HAAN, I., and SCHOOREL, A. F. (1940). *Arch. Theecult.* **14** : 43. Kaligebrek in de theecultur. 5, 7, T19B, 107, 143, 152

HAAS, A. R. C. (1929). *Bot. Gaz.* **88** : 113. Toxic effect of boron on fruit trees. 112

HAAS, A. R. C. (1930). *Bot. Gaz.* **89** : 410. Boron as an essential element for healthy growth of citrus. 18, 143

HAAS, A. R. C. (1932a). *Hilgardia* **6** : 483. Some nutritional aspects in " mottle leaf " in citrus. 15, 87, T19B, 99, 113, 131, 135, 143

HAAS, A. R. C. (1932b). *Hilgardia* **7** : 181. Injurious effects of manganese and iron deficiencies on the growth of citrus. 15, 66, 112, 122, 135, 143, 160

HAAS, A. R. C. (1937). *Plant Physiol.* **12** : 163. Nitrogen in relation to the growth of citrus cuttings in solution culture. 12, 15, T19B, 143

HAAS, A. R. C. (1940). *Plant Physiol.* **15** : 377. Relation of pH to growth of citrus. 94, 143

HAAS, A. R. C. (1946). *Soil Sci.* **60** : 445. Influence of the rootstock on the composition of citrus leaves and rootlets. 143, 165

HAAS, A. R. C. (1949). *Plant Physiol.* **24** : 395. Potassium in citrus trees. 8, 18, 113, 143

HAAS, A. R. C., and KLOTZ, L. J. (1931). *Bot. Gaz.* **92** : 94. Further evidence of the necessity of boron for health in citrus. 18, 22, 143

HAAS, A. R. C., and REED, H. S. (1927). *Bot. Gaz.* **83** : 77. Significance of traces of elements not ordinarily added to culture solutions for the growth of young orange trees. 109, 143

HAAS, A. R. C., see REED, H. S.

HAGEMAN, R. H., MCHARGUE, J. S., SHERMAN, G. D., and HODGE, E. S. (1942). *J. Amer. Soc. Agron.* **34** : 731. The production of " grey speck " of oats in purified sand cultures. 21, 23, 31, 51, 75, 82

HAGEMAN, R. H., see HELLER, V. G.

HAHN (1922) given by ROBERG (1931). 54

HALL, A. D., BRENCHLEY, W. E., and UNDERWOOD, L. M. (1914a). *Phil. Trans. roy. Soc.* (*London*) B. **204** : 179. The soil solution and mineral constituents of the soil. 3, 26, 27, 154

HALL, A. D., BRENCHLEY, W. E., and UNDERWOOD, L. M. (1914b). *J. agric. Sci.* **6** : 278. The soil solution and the mineral constituents of the soil. 3, 26, 27, 154

HALLAS, D. G., see HEWITT, E. J.

HAMNER, C. L. (1940a). *Bot. Gaz.* **101** : 637. Growth responses of Biloxi soya beans to variation in relative concentrations of phosphate and nitrate in the nutrient solutions. 167

HAMNER, C. L. (1940b). *Bot. Gaz.* **102** : 156. Effects of vitamin B_1 upon the development of some flowering plants. 115

HAMNER, K. C., see VOTH, P. O.

HAMPE, W. (1868). *Landw. VersSta.* **10** : 175. Vegetationsversuche mit Ammoniaksalzen, Harnsäure, Hippursäure und Glycocoll als stickstoffhaltigen Nahrungsmitteln der Pflanzen. 93, T19A, 97

HARRIS, T. M. (1946). *New Phytol.* **45** : 50. Zinc poisoning of wild plants from wire netting. 46

HARRISON, J. W. E., MYERS, R. J., and HERR, D. S. (1943). *J. Amer. pharm. Ass.* **32** : 121. Purified mineral free water for pharmaceutical purposes. 57

HART, C. E. (1934). *Plant Physiol.* **9** : 399. Some effects of potassium upon the growth of sugar cane and upon the absorption and migration of ash constituents. 153

HARTMAN, E. L., see HELLER, V. G. ; WALL, R. F.

HARTWELL, B. L., and PEMBER, F. R. (1908). *A.R. Rhode Island agr. Exp. Stat.* **21** p. 243. Sodium as a partial substitute for potassium. 92

HARTWELL, B. L., and PEMBER, F. R. (1918). *Soil Sci.* **6** : 259. The presence of aluminium as a reason for the difference in the effect of so-called acid soil on barley and rye. T19A, 104

HARTWELL, B. L., WHEELER, H. J., and PEMBER, F. R. (1907). *A.R. Rhode Island agric. Exp. Stat.* **20**, p. 299. The effect of the addition of sodium to deficient amounts of potassium upon the growth of plants in both water and sand cultures. 92, T19A

HASKINS, C. P., see HUTNER, S. H.

HATCHER, J. T., see AYERS, A. D.

V. HAUSEN, S. S. (1948). *Physiol. Plant.* **1** : 85. On the rôle of growth substances in higher plants. 115, 164

V. HAUSEN, S. S., see VIRTANEN, A. I.

HAYNES, J. L., and Robbins, W. R. (1948). *J. Amer. Soc. Agron.* **40** : 707. Calcium and boron as essential factors in the root environment. 25

HAYWARD, H. E., and LONG, E. M. (1941a). *Bot. Gaz.* **102** : 437. Anatomical and physiological responses of the tomato to varying concentrations of sodium chloride, sodium sulphate and nutrient solutions. 18, 91

HAYWARD, H. E., and LONG, E. M. (1941b). *Proc. Amer. Soc. hort. Sci.* **41** : 149. Vegetative responses of the Elberta peach on Lovell and Shalil rootstocks to high chloride and sulphate solutions. 18, 50, 91, T19B, 99, 102, 141, 145

HAYWARD, H. E., and LONG, E. M. (1943). *Plant Physiol.* **18** : 556. Some effects of sodium salts on the growth of the tomato. 49, 91, T19B, 114

HAYWARD, H. E., LONG, E. M., and UHVITS, R. (1946). *Tech. Bull. U.S. Dep. Agric.* **922**. Effect of chloride and sulphate salts on the growth and development of the Elberta peach on Shalil and Lovell rootstocks. 8, 9, 18, 19, 28, 31, T7, 48, 49, T19B, 102, 110, 114, 141, 143, 145, 147

HAYWARD, H. E., and SPUR, W. B. (1944). *J. Amer. Soc. Agron.* **36** : 287. The tolerance of flax to saline conditions. Effect of sodium chloride, calcium chloride, and sodium sulphate. 91, T19B, 108

HEATH, O. V. S. (1929). *Ann. Bot. Lond.* **43** : 71. A method of water control for sand cultures. 124

HEATH, O. V. S., and GREGORY, F. G. (1938). *Ann. Bot. Lond.* **2**, n.s. : 811. The constancy of the mean nett assimilation rate and its ecological importance. 168

HEATH, O. V. S., and WHITCHER, E. J. (1945). *Ann. appl. Biol.* **32** : 173. A simple automatic window opening device for temperature control in greenhouses. 161

HECK, W. W., and BAILEY, L. F. (1950). *Plant Physiol.* **25** : 573. Chelation of trace metals in nutrient solutions. 80

HELLER, V. G., HAGEMAN, R. H., and HARTMAN, E. L. (1940). *Plant Physiol.* **15** : 727. Sand culture studies of the use of saline and alkaline waters in greenhouses. 91, 118

VAN HELMONT. 2

HELRIEGEL (1898) given by PFEFFER (1918). 31, T7, 156

HELRIEGEL and WILFARTH (1898). 92

HENDERSON, F. Y., see ASHBY, E.

HENDERSON, J. H. M., and VEAL, M. P. (1948). *Plant Physiol.* **23** : 609. The effect of the interrelationships of boron and manganese on the growth and calcium uptake of blue lupin (*Lupinus angustifolius* L.) in solution culture. 112

HENDRIKS, R. H., see THOMAS, L. S.

HENDRICKS, S. B., see HOPKINS, H. T.

HENRY, V. M., see TURNER, W. I.

HERR, D. S., see HARRISON, J. W. E.

HEWITT, E. J. (unpublished data). 15, 20, 24, T4, 28, 30, T7 (33), 41, 45, 47, 48, 54, 59, 64, 65, 66, 70, 72, 78, 81, 82, 92, 95, 97, 98, 105, 106, 111, 112, 114, 150, 159, 160, 163

HEWITT, E. J. (1944). *A.R. Long Ashton agric. hort. Res. Stat.* 1943, p. 33. Experiments in mineral nutrition I. The visual symptoms of mineral deficiencies in vegetables and cereals grown in sand cultures. Progress report 1943. 5, 7, 19, 32, 48

HEWITT, E. J. (1945). *A.R. Long Ashton agric. hort. Res. Stat.* 1944, p. 50. Experiments in mineral nutrition II. The visual symptoms of mineral deficiencies in crop plants grown in sand cultures. Progress report 1944. 5, 7, 19, T4, 32, 41, 48, 51, 75

HEWITT, E. J. (1946a). *A.R. Long Ashton agric. hort. Res. Stat.* 1945, p. 44. Experiments in mineral nutrition III. The visual symptoms of mineral deficiencies in crop plants grown in sand cultures. Progress report 1945. 5, 7, 20, T4, 29, 31, 32, 48, 51, 57, 70, 71, 75

HEWITT, E. J. (1946b). *A.R. Long Ashton agric. hort. Res. Stat.* 1945, p. 51. The resolution of the factors in soil acidity. Progress report I. 1945. 5, 7, 20, T4, 32, 48, 112

HEWITT, E. J. (1946c). *Nature,* **158** : 623. Use of water purified by synthetic resin ion-exchange methods for the study of mineral deficiencies in plants. 57

HEWITT, E. J. (1947a). *A.R. Long Ashton agric. hort. Res. Stat.* 1946, p. 37. A technique for large-scale pot sand cultures. 5, 20, 29, 31, 32, 51, 57, 70, 71, 75, 176

HEWITT, E. J. (1947b). *A.R. Long Ashton agric. hort. Res. Stat.* 1946, p. 50. The resolution of the factors in soil acidity : some effects of manganese toxicity. 5, 7, 20, 41, 48, 95, 98, 112

HEWITT, E. J. (1948a). *A.R. Long Ashton agric. hort. Res. Stat.* 1947, p. 82. The resolution of the factors in soil acidity : the relative effects of aluminium and manganese toxicities in farm and market garden crops. 5, 7, 20, 41, 48, 98, 112

HEWITT, E. J. (1948b). *Nature,* **161** : 489. Relation of manganese and some other metals in the iron status of plants. 108, 111, 114

HEWITT, E. J. (1949a). *A.R. Long Ashton agric. hort. Res. Stat.* 1948, p. 66. Experiments in iron metabolism in plants I. Some effects of metal-induced iron deficiency. 20, 48, 102, 108, 111, 114

HEWITT, E. J. (1949b). *A.R. Long Ashton agric. hort. Res. Stat.* 1948, p. 58. The resolution of the factors in soil acidity. The comparative effects of manganese and aluminium toxicities on some farm and market garden crops (continued). 20, 29, 48

HEWITT, E. J. (1949c). *Brit. Sci. News* **2** : 254. The relation of manganese and other metal toxicities to the iron status of plants. 108, 111, 114

HEWITT, E. J., AGARWALA, S. C., and JONES, E. W. (1950). *Nature,* **166** : 1119. The effect of molybdenum status on the ascorbic acid content of plants in sand culture. 116

HEWITT, E. J., and HALLAS, D. G. (1951). *Plant and Soil* **3** : 366. The use of *Aspergillus niger* (Van Tiegh.) M strain as a test organism in the study of molybdenum as a plant nutrient. 23, 33, 51, 54, 55, 65, 66, 67, 68, 71, 73, 76, 79, 80, T18, 192, 196

HEWITT, E. J., and JONES, E. W. (BOLLE-JONES) (unpublished). 23, 51, 54, 80, 114, 153, 164, 191, 192

HEWITT, E. J., and JONES, E. W. (1947). *J. Pomol.* **23** : 254. The production of molybdenum deficiency in plants in sand culture with special reference to tomato and *Brassica* crops. 5, 7, 22, 23, 54, 71, 78, 79, 81, 82, 194

HEWITT, E. J., and JONES, E. W. (1949). *A.R. Long Ashton agric. hort. Res. Stat.* 1948, p. 81. Molybdenum as a plant nutrient. The effects of molybdenum deficiency on some vegetables, cereals and forage crops. 5, 7, 22, 23, 54, 71, 78, 79, 81, 194

HEWITT, E. J., and JONES, E. W. (1950). *A.R. Long Ashton agric. hort. Res. Stat.* 1949, p. 58. Molybdenum as a plant nutrient. II. Effect of molybdenum deficiency on some *Brassica* crops. 7, 22, 54, 71, 79, 81, 192, 194

HEWITT, E. J., and JONES, E. W. (1951). *A.R. Long Ashton agric. hort. Res. Stat.* 1950, p. 56. The effect of zinc and copper deficiencies on crop plants, grown in sand culture. 7, 22, 54

HEWITT, E. J., and JONES, E. W. (1952). *J. hort. Sci.* (in the Press). Molybdenum as a plant nutrient. I. The influence of molybdenum on the growth of some *Brassica* crops in sand culture. 79, 81

HEWITT, E. J., see WALLACE, T.

HIBBARD, P. L., see HOAGLAND, D. R.

HIBBARD, R. P., and GERSHBERG, S. (1924). *Tech. Bull. Mich. agric. Exp. Stat.* **64**. The salt requirements of Marquess wheat in water culture for the vegetative phase of development. T19B, 151, 152, 165

HICKS, F., and TINCKER, M. A. H. (1944). *J. roy. hort. Soc.* **69** : 112. Growing carnations in sand. T4, 28, 40

HILL, G. R., see THOMAS, L. S.

HILL, H., and GRANT, E. P. (1935). *Sci. Agric.* **15** : 652. The growth of turnips in artificial cultures. 67

HILL, H., and JOHNSTON, F. B. (1940). *Sci. Agric.* **20** : 9. Magnesium deficiency of apple trees in sand culture and in commercial orchards. 8, T19B, 141

HILL, H., see DAVIS, M. B.

HILL, W. L., see RADER, L. F.

HILLEBRAND, W. F., and LUNDELL, G. E. F. (1929). "Applied inorganic analysis." Wiley, New York, Chapman & Hall, London. 81

HOAGLAND, D. R. (1917). *Soil Sci.* **3** : 547. The effect of hydrogen and hydroxyl ion concentration on the growth of barley seedlings. 98, 100

HOAGLAND, D. R. (1919). *J. agric. Res.* **18** : 73. Relation of concentration and reaction of the nutrient medium to the growth and absorption of the plant. 3, 5, 7, 16, T4, 27, 31, 89, 90, T19A, 105, 139

HOAGLAND, D. R. (1937). *Bot. Rev.* **3** : 307. Some aspects of the salt nutrition of higher plants. 93, 95

HOAGLAND, D. R. (1941). *Proc. Amer. Soc. hort. Sci.* **38** : 8. Water culture experiments on molybdenum and copper deficiencies of fruit trees. 11, 17, 22, 75, 76, 108, 110, 113, 143, 150

HOAGLAND, D. R. (1944). "Lectures on the inorganic nutrition of the higher green plants." Chronica Botanica Co., Waltham Mass. 90, 159

HOAGLAND, D. R., and ARNON, D. I. (1939). *Circ. Calif. agric. Exp. Stat.* **347** and *Rep. Smithsonian Inst. 1938*, p. 461. Miscellaneous publication No. 3514. The water culture method for growing plants without soil. 15, 16, 18, 19, 135, 147, 151

HOAGLAND, D. R., and ARNON, D. I. (1941). *Soil Sci.* **51** : 431. Physiological aspects of the availability of nutrients for plant growth. 88, 92

HOAGLAND, D. R., and BROYER, T. C. (1936). *Plant Physiol.* **11** : 471. General nature of the process of salt accumulation by roots with description of experimental methods. 14, 15, 16, 18, 131, 133, 137, 139, 140, 152, 156

HOAGLAND, D. R., CHANDLER, W. H., and HIBBARD, P. L. (1935). *Proc. Amer. Soc. hort. Sci.* **33** : 131. Little leaf or rosette of *Citrus* V. Effect of zinc on growth of various types in controlled soil and water culture experiments. 11, 12, 16, 71

HOAGLAND, D. R., and MARTIN, J. C. (1923). *Soil Sci.* **16** : 367. A comparison of sand and solution cultures with soils as media for plant growth. 6, 84, 87, 155

HOAGLAND, D. R., and SNYDER, W. C. (1933). *Proc. Amer. Soc. hort. Sci.* **30** : 288. Nutrition of strawberry plant under controlled conditions. (a) Effects of deficiencies of boron and certain other elements, (b) Susceptibility to injury from sodium salts. 12, 16, 36, 67, 85, 92, T19A, 108, 109, 114, 115, 150

HOAGLAND, D. R., see ARNON, D. I. ; DAVIS, A. R. ; JOHNSTON, E. S.

HOBBS, C. H. (1944). *Plant Physiol.* **19** : 590. Studies on mineral deficiency in pine. 29, T7, T19B

HODGE, E. S., see HAGEMAN, R. H.

HOENER, I. R., and DE TURK, E. E. (1938). *J. Amer. Soc. Agron.* **30** : 232. The absorption and utilisation of nitrate nitrogen during vegetative growth by Illinois high-protein and Illinois low-protein corn. 165

HOFFER, G. N. (1926). *J. Amer. Soc. Agron.* **18** : 322. Some differences in the functioning of selfed lines of corn under varying nutritional conditions. 165

HOFFMAN, W., see ARNDT, TH.

HOLMES, E. L., see ADAMS, B. A.

HOLMES, F. E. (1940). *Industr. Engng. Chem. (Anal.)* **12** : 483. Apparatus for maintaining constant levels in a water bath and a distilling flask. 55

HOLT, M. E., and VOLK, N. J. (1945). *J. Amer. Soc. Agron.* **37** : 821. Sodium as a plant nutrient and substitute for potassium. 92, 102

HONE, M. R., see WOOD, J. G.

HOOPER, M. C. (1937). *Ann. appl. Biol.* **24** : 690. An investigation of the effect of lead on plants. 147

HOOVER, S. R., see HORNER, C. K.

HOPKINS, E. F. (1934). *Mem. Cornell agric. Exp. Stat.* **151**. Manganese, an essential element for green plants. 67, 68, 71, 74, 149, 154

HOPKINS, E. F., and WANN, F. B. (1927). *Bot. Gaz.* **84** : 107. Iron requirements for *Chlorella*. 74, 103

HOPKINS, E. F., PAGAN, V., and RAMIREZ-SILVA, F. J. (1944). *J. Agric. Univ. Puerto Rico* **28** : 143. Iron and manganese in relation to plant growth and its importance in Puerto Rico. 108

HOPKINS, E. W., WILSON, P. W., and FRED, E. B. (1931). *J. Amer. Soc. Agron.* **23** : 32. A method for the growth of leguminous plants under bacteriologically controlled conditions. 149

HOPKINS, H. T., SPECHT, A. W., and HENDRICKS, S. B. (1950). *Plant Physiol.* **25** : 193. Growth and nutrient accumulation as controlled by oxygen supply to plant roots. 133, 137

HORNER, C. K., and BURK, D. (1934). *J. agric. Res.* **48** : 981. Magnesium, calcium and iron requirements for growth of *Azotobacter* in free and fixed nitrogen. 66, 75

HORNER, C. K., BURK, D., ALLISON, F. E., and SHERMAN, M. S. (1942). *J. agric. Res.* **65** : 173. Nitrogen fixation by *Azotobacter* as influenced by molybdenum and vanadium. 68, 73, 76

HORNER, C. K., BURK, D., and HOOVER, S. R. (1934). *Plant Physiol.* **9** : 663. Preparation of humate iron and other humate metals. 106, 114

HORNER, G. H. (1936). *Res. Bull. Mo. agric. Exp. Stat.* **232**. Relation of the degree of base saturation of a colloidal clay by calcium to the growth, nodulation and composition of soya beans. 35, 38, 39, 141

HOUGHLAND, G. V. C. (1947). *J. agric. Res.* **75** : 1. Minimum phosphate requirement of potato plants in culture solutions. 12, 16, 21, 48, 87, T19B, 108, 133, 154, 164

HOUGHLAND, G. V. C. (1950). *Amer. Potato J.* **27** : 257. An improved technique for growing potatoes in solution cultures. 13, 17, 133, 135

HOWARD, F., and SPOONER, E. C. R. (1946). *Chem. Industr. Lond.* March 9th 1946. Lecture to Food Group, Feb. 4th 1946. " The removal of taints from water." 62

HOWARD, J. N., see KENWORTHY, A. L.

HUBBEL, D. S. (1930). *Proc. Amer. Soc. hort. Sci.* **27** : 475. The influence of various plant containers on the growth and development of *Pelargonium hortorum*. 14

HUGHES, R. C. (1943). *J. opt. Soc. Amer.* **33** : 49. Preparation of purified inorganic compounds for use in spectrographic standards. T7, 70, 71, 72, 76, 80

HUMPHRIES, E. C. (1950). *J. exp. Bot.* **1** : 282. The absorption of ions by excised root systems. I. Apparatus and preliminary measurements. 42, 124, 130, 141

HUTCHINSON, H. B., and MILLER, N. H. J. (1909). *J. agric. Sci.* **3** : 179. Direct assimilation of ammonium salts by plants. 149

HUTCHINSON, H. B., and MILLER, N. H. J. (1912). *J. agric. Sci.* **4** : 282. The direct assimilation of inorganic and organic forms of nitrogen by plants. 93, 97

HUTNER, S. H., PROVASOLI, L., SCHATZ, A., and HASKINS, C. P. (1950). *Proc. Amer. phil. Soc.* **94** : 152. Some approaches to the study of the rôle of metals in the metabolism of microorganisms. 24, 54, 66, 71, 72, 82

s'JACOB, J. C. (1938). *Arch. Koffiecult.* **12** : 1. Voedingsphysiologische onderzoekingen bij *Coffea arabica* I. 143

s'JACOB, J. C. (1939). *Arch. Koffiecult.*, **13** : 27. Voedingsphysiologische onderzoekingen bij *Coffea arabica*. II. De stikstofvoeding. 11, 99, 107, 108, 143

JACOBSEN, H. G. M., and SWANBACK, T. R. (1929). *Science* **70** : 283. Manganese toxicity in tobacco. 112, 155

JACOBSEN, H. G. M., and SWANBACK, T. R. (1933). *Plant Physiol.* **8** : 340. Relative influence of NO_3 and NH_4+N upon the uptake of Ca by tobacco plants. 95

JACOBSEN, L. (1951). *Plant Physiol.* **26** : 411. Maintenance of iron supply in nutrient solutions by a single addition of ferric potassium ethylene diamine tetra acetate. 107

JACOBSEN, L., see STOUT, P. R.

JANSSEN, G., and BARTHOLOMEW, R. P. (1930). *J. agric. Res.* **40** : 243. The influence of potash concentration in the cultural medium on the production of carbohydrate in plants. 87

JANSSEN, G., see BARTHOLOMEW, R. P.

JAULMES, P., and GALHAC, E. (1937). *Bull. Soc. chim. Fr.* (Ser. V) **4** : 135. Sur la volatilité des esters boriques en solution alcoholique. 72

JAVILLIER, M. (1907). *C.R. Acad. Sci.* (*Paris*) **145** : 1212. Sur l'influence favorable de petites doses de zinc sur la végétation du *Sterigmatocystis nigra* (Van Tigh.). 3, 54

JAVILLIER, M. (1914). *C.R. Acad. Sci.* (*Paris*) **158** : 140. Une cause d'erreur dans l'étude de l'action biologique des éléments chimiques ; la présence de traces de zinc dans le verre. 23

JAVILLIER, M., see BERTRAND, G.

JENNY, H. (1946). *J. Colloid Sci.* **1** : 33. Adsorbed nitrate ions in relation to plant growth. 39

JENNY, H., and COWAN, E. W. (1933). *Science* **77** : 394. The utilisation of adsorbed ions by plants. 39

JENNY, H., and OVERSTREET, R. (1938). *Proc. nat. Acad. Sci. Wash.* **24** : 384. Contact effects between plant roots and soil colloids. 39

JENNY, H., and OVERSTREET, R. (1939). *Soil Sci.* **47** : 257. Cation interchanges between plant roots and soil colloids. 39

JENNY, H., OVERSTREET, R., and AYERS, A. D. (1939). *Soil Sci.* **48** : 9. Contact depletion of barley roots as revealed by radio-active indicators. 39

JENSEN, G. H. (1907). *Bot. Gaz.* **34** : 11. Toxic limits and stimulation effects of some salts and poisons. 155

JENSEN, H. L., and BETTY, R. L. (1943). *Proc. Linn. Soc. N.S. Wales* **68** : 1. Nitrogen fixation by leguminous plants III. The importance of molybdenum in symbiotic nitrogen fixation. 29, T7, 50, 164

JOHNSON, C. M., and ATKIN, S. (1939). *Plant Physiol.* **14** : 391. An automatic plant irrigator and recorder. 122

JOHNSON, C. M., see ARNON, D. I.

JOHNSON, F. B., see DAVIS, M. B.

JOHNSTON, B., see FURNSTALL, A. H.

JOHNSTON, E. S. (1920). *Soil Soc.* **10** : 389. Nutrient requirements of the potato plant grown in sand cultures treated with type I solutions. 5, 6, 7, 137, 153, 166

JOHNSTON, E. S. (1921). *Bull. Md agric. Exp. Stat.* **245**, p. 41. The seasonal march of the climatic conditions of a greenhouse as related to plant growth. 151, 161, 166

JOHNSTON, E. S. (1924). *Bull. Md agric. Exp. Stat.* **270**, p. 51. Growth of potato plants in sand cultures treated with the " Six types " of nutrient solutions. 90, 164, 167

JOHNSTON, E. S. (1925). *Soil Sci.* **20** : 397. Comparative study of the " Six types " of nutrient solutions in relation to the growth of potato plants in sand cultures. 137

JOHNSTON, E. S. (1927). *Plant Physiol.* **2** : 213. An apparatus for controlling the flow of nutrient solutions in plant cultures. 120, 124, 125, 126

JOHNSTON, E. S., and HOAGLAND, D. R. (1929). *Soil Sci.* **27** : 86. Minimum potassium level required by tomato plants grown in water cultures. 13, 16, 86, 87, 88, 90, 159

JOHNSTON, F. B., see HILL, H.

JONES, C. D., and SKINNER, C. E. (1927). *A.R. New Jersey agric. Exp. Stat.* 1926, p. 360. Absorption of nitrogen from culture solutions by plants. 13, 95, 139

JONES, E. W. (unpublished data). 4, 187, 199

JONES, E. W., see HEWITT, E. J.

JONES, L. H. (1920). *A.R. New Jersey agric. Exp. Stat.* 1919, p. 405. The effect of ammonium sulphate upon the availability of iron in nutrient solutions. 95, 103

JONES, L. H., SHEPARDSON, W. B., and PETERS, C. A. (1949). *Plant Physiol.* **24** : 300. The function of manganese in the assimilation of nitrates. 138

JONES, L. H., and SHIVE, J. W. (1921a). *Soil Sci.* **11** : 93. The influence of iron in the form of ferric phosphate and ferrous sulphate on the growth and absorption of the plants. T19A, 103

JONES, L. H., and SHIVE, J. W. (1921b). *J. agric. Res.* **21** : 701. Effect of ammonium sulphate upon plants in nutrient solutions supplied with ferric phosphate and ferrous sulphate as sources of iron. 95, T19A, 103

JONES, L. H., and SHIVE, J. W. (1923). *Ann. Bot. Lond.* **37** : 355. Influence of ammonium sulphate on plant growth in nutrient solutions and its effect on hydrogen ion concentration and iron availability. 103

JONES, L. R. (1922). *Trans. Wis. Acad. Arts Lett.* **20** : 433. Experimental work on the relation of soil temperature to disease in plants. 161

JÖRGENSEN, I., see STILES, W.

KALIN, E. W. (1943). *Proc. Amer. Soc. hort. Sci.* **43** : 235. pH of extracted cell sap and phosphorus content of young tomato plants growing on varying levels of phosphorus. 31, 87

KAMEN, M. D. (1947a). *Ann. Rev. Biochem.* **16** : 631. Use of isotopes in biochemical research. 117

KAMEN, M. D. (1947b). " Radioactive tracers in biology. Introduction to tracer methodology." Organic and biological chemical monographs Vol. 1, Academic Press, New York. 117

KARRAKER, P. E., see BORTNER, C. E.

KARSTROM, H., see VIRTANEN, A. I.

KENWORTHY, A. L. (private communication). 18, 30, T7, 57

KENWORTHY, A. L., and HOWARD, J. N. (1944). *Soil Sci.* **57** : 293. Purification of water by use of synthetic ion-exchange resins using pH as a control. 62

KENYON, A. E., see Street, H. E.

KHARAK-SING, see BRENCHLEY, W. E.

KIDD, F., and WEST, C. (1918). *Ann. appl. Biol.* **5** : 1, 112, 157, 220. Physiological predetermination. The influence of the physiological conditions of the seed upon the course of subsequent growth and on the yield. I. The effects of soaking the seeds in water, II-IV. Review of literature. 164, 165

KIDD, F., see BRIGGS, G. E. ; WEST, C.

KING, A. (1933). *J. chem. Soc. Lond.* p. 842. Studies in chemisorption on charcoal. I. The acid constituents of charcoal. 72

KING, A. (1934a). *J. chem. Soc. Lond.* p. 22. II. Experiments on the formation of the acid constituents of charcoal. 72

KING, A. (1934b). *J. chem. Soc. Lond.* p. 1975. V. The influence of ultra porosity on adsorption. 72

KING, A. (1937). *J. chem. Soc. Lond.* p. 1489. The influence of temperature of activation on the sorption of acids and bases. 72

KING, A., see BENNISTER, H. L.

KIPLINGER, D. C., see LAURIE, A.

KLEIN, G. (1930). Ergebnisse der AgrikChem. (*Jb. landw. Chem.*) **11** : 143. Der Wandel des Stickstoffs in der grünen Pflanze. 150

KLOTZ, L. G. (1944). *Phytopathology* **34** : 507. A simplified method of growing plants with roots in nutrient vapours. 122

KLOTZ, L. J., see HAAS, A. R. C.

KNOP, W. 89

KNOP, W. (1860). *Landw. VerstSta.* **2** : 65, 270. Ueber die Ernährung der Pflanzen durch wässerige Lösungen bei Ausschluss des Bodens. 2, 42, 151

KNOP, W. (1861). *Landw. VersSta.* **3** : 295. Quantitative analytische Arbeiten über den Ernährungsprocess der Pflanzen. 2, 83, T19A, 103, 106

KNOP, W. (1865). *Landw. VersSta.* **7** : 93. Quantitative Untersuchungen über die Ernährungsprocesse der Pflanzen. 2, 3, 84, T19A, 106, 107

KNOP, W. W., see WOLFE, E.

KOLODNY, L., and ROBBINS, W. R. (1940). *Soil Sci.* **49** : 303. Availability of fixed potassium to plants. 31, 39, 124

KRASSOVSKY, I. (1926). *Soil Sci.* **21** : 307. Physiological activity of the seminal and nodal roots of crop plants. 25

KRAUSE, A., see NIKLEWSKI, B.

KRAUSS, B. H., see SIDERIS, C. P.

LAGASSE, F. S. (1929). *Proc. Amer. Soc. hort. Sci.* **26** : 187. Some growth responses of Elberta peach trees to fertiliser treatments. 6, 8, 18, 141, 145

LARSINOS, G. J., see BEAUMONT, A. B.

LAURIE, A., and KIPLINGER, D. C. (1940). *Bull. Ohio agric. Exp. Stat.* **616**. Growing ornamental greenhouse crops in gravel culture. 26, 143

LAWES, J. B., GILBERT, J. H., and WARINGTON, R. (1881). *J. roy. agric. Soc.* **17** : 241. On the amount and composition of rain and drainage waters collected at Rothamsted. 44

LAWRENCE, W. J. C. (1948). "Science and the glasshouse." Oliver and Boyd, Edinburgh. 159

LAWRENCE, W. J. C., and NEWELL, J. (1941). "Seed and potting composts." 2nd Edit. Allen and Unwin, London. 198

LEAVENWORTH, C. S., see PUCHER, G. W.

LEBEDEFF, A. F. (1927). *Proc. 1st Internat. Congr. Soil Sci.* **1** : 459. The movement of ground and soil waters. 6, 27, 28

LECLERG, E. L. (1935). *Phytopathology* **25** : 1019. Factors affecting experimental error in greenhouse pot tests with sugar beets. 166

LEHR, J. J. (private communication). 15

LEHR, J. J. (1940). De Betekenis van Borium voor de Plant. Thesis Utrecht. 11, 15, 16, 17, 64, 65, 66, 67, 68, T19B, 109, 111, 135, 151

LEHR, J. J. (1941). *Soil Sci.* **52** : 373. The importance of sodium for plant nutrition. II. The effect on beets of the secondary ions in nitrate fertilizers. 38, 92, 93

LEHR, J. J. (1942). *Soil Sci.* **53** : 399. Importance of sodium for plant nutrition. III. Equilibrium of cations in beet. 36, 38, 92

LEHR, J. J. (1947). *Soil Sci.* **63** : 479. The importance of sodium for plant nutrition. IV. Influence of nitrate fertilizers on the equilibrium of cations in fodder beet. 38, 92

LEIGHTON, W. G., and FORBES, G. S. (1930). *J. Amer. chem. Soc.* **52** : 3139. Precision actinometry with uranyl acetate. 163

LEMANEZYK, E., see NIKLEWSKI, B.

LEMMERMANN, O., and WEISMANN, H. (1922). *Z. PflErnähr. Düng* A **1** : 185. Die ertragsteigernde Wirkung der Kieselsäure bei unzureichender Phosphorsäureernährung der Pflanzen. 40

LEUKEL, R. W. (1924). *Phytopathology* **14** : 384. Equipment and methods for studying the relation of soil temperature to diseases in plants. 161

LEWIS, A. H. (1936). *J. agric. Sci.* **26** : 249. The effects of ammonium and nitrate nitrogen on the growth of perennial rye-grass. 39, 96

LEWIS, J. C., and POWERS, W. L. (1941). *Plant Physiol.* **16** : 393. Antagonistic action of chlorides on the toxicity of iodides to corn. 48, 50, T19B, 107, 110

LIEBIG, G. F. Jr. (1940). *Industr. Engng Chem. (Anal.)* **12** : 174. An all-glass still with automatic float feed. 54, 55

LIEBIG, G. F. Jr., VANSELOW, A. P., and CHAPMAN, H. D. (1942). *Soil Sci.* **53** : 341. Effects of aluminium on copper toxicity as revealed by solution cultures and spectrographic studies of citrus. 11, 51, 75, T19B, 112, 113, 119, 137, 143, 152

LIEBIG, G. F. Jr., VANSELOW, A. P., and CHAPMAN, H. D. (1943a). *Soil Sci.*, **55** : 371. The suitability of water purified by synthetic ion-exchange resins for the growing of plants in controlled nutrient cultures. 49, 50, 51, 57, 143

LIEBIG, G. F. Jr., VANSELOW, A. P., and CHAPMAN, H. D. (1943b). *Soil Sci.* **56** : 173. Effects of gallium and indium on the growth of citrus plants in solution cultures. 4, 11, 16, 17, 76, 111, 113, 137, 143, 152

LIEBIG, G. F. Jr., see CHAPMAN, H. D.

LINEBERRY, R. A., and BURKHART, L. (1943). *Plant Physiol.* **18** : 325. Nutrient deficiencies in strawberry leaf and fruit. 8, T19B, 113

LINGANE, J. J., and MEITES, L. Jr. (1946). *J. Amer. chem. Soc.* **68** : 2443. Extraction of vanadium from aqueous solutions by isopropyl ether. 81

LINK, G. K. K. (1935). *Science* **81** : 204. The Chicago soil nutrient temperature tank. 161

LINKOLA, H., see VIRTANEN, A. I.

LIPMAN, C. B. (1938). *Soil Sci.* **45** : 189. The importance of silicon, aluminium and chlorine for higher plants. 4, 22, 109, 164

LIPMAN, C. B., and MACKINNEY, G. (1931). *Plant Physiol.* **6** : 593. Proof of the essential nature of copper for higher green plants. 4, 11, 22, 70

LIPMAN, C. B., see SOMMER, A. L.

LIPMAN, J. G. (1910). *J. agric. Sci.* **3** : 297. A method for the study of soil fertility problems. 24

LIPMAN, J. G. (1912). *Bull. New Jersey agric. Exp. Stat.* **253**. The associative growth of legumes and non-legumes. 24

LIVINGSTONE, B. E. (1942). *Publ. Amer. Ass. Adv. Sci.* **16**, pp. 83-111. Mineral requirements of plants as indicated by means of solution cultures. 83, 90, 98

LIVINGSTONE, B. E., and TOTTINGHAM, W. E. (1918). *Amer. J. Bot.* **5** : 337. A new three-salt nutrient solution for plant cultures. 3, 83, 88, T19A, 166

LIVINGSTONE, B. E., see GROSSENBACHER, K. A.; TRELEASE, S. F.

LOHMAN, G. (1934). *Arch. Mikrobiol.* **5** : 31. Nährstoffwirkung und Giftwirkung bei *Aspergillus niger*. 73

LÖHNIS, M. P. (1937). *Meded. LandbHoogesch. Wageningen* **41**, (part 3). Plant development in the absence of boron. 10, 15, 16, 22, 29, 50, 67, 109, 114, 151, 164

LÖHNIS, M. P. (1944). *Antonie van Leeuwenhoek* **10** : 101. The action of manganese on the development of *Aspergillus niger*. 66, 73, 74

LONG, E. M., see HAYWARD, H. E.

LOO, T. L. (1928). *Jap. J. Bot.* **4** : 71. The effect of renewal of nutrient solutions on the growth of culture plants and its relation to aeration. 100

LOOMIS, W. E., see CHANG, H. T.

LOOSJES, R., see SCHUFFELEN, A. C.

LOUSTALOT, A. J., GILBERT, S. G., and DROSDOFF, M. (1950). *Plant Physiol.* **25** : 394. The effect of nitrogen and potassium levels in tung seedlings on growth, apparent phytosynthesis, and carbohydrate composition. 8, 57

LOUSTALOT, A. J., see CIBES, H. R.

LOW, A. J. (1948). *Agriculture* **55** : 210. Fluorescent lighting as an aid to winter research. 163

LUCANUS, B., see BIRNER, H.

LUNDELL, G. E. F., see HILLEBRAND, W. F.

LYNESS, A. S. (1936). *Plant Physiol.* **11** : 665. Varietal difference in the phosphorus feeding capacity of plants. 165

MACHLIS, L. (1941). *Plant Physiol.* **16** : 521. Accumulation of arsenic in the shoots of Sudan grass and bush bean. 151, 166, 169

MACINTYRE, W. H., and YOUNG, J. B. (1923). *Soil Sci.* **15** : 205. Sulphur, calcium, magnesium and potassium content and reaction of rainfall at different points in Tennessee. 42, 43, 44

MACKINNEY, G., see LIPMAN, C. B.

MACVICAR, R., and STRUCKMEYER, B. E. (1946). *Bot. Gaz.* **107** : 454. The relation of photoperiod to the boron requirement of plants. 20, 159

MAGISTAD, O. C., AYERS, A. D., WADLEIGH, C. H., and GAUCH, H. G. (1943). *Plant Physiol.* **18** : 151. Effect of salt concentration, kind of salt and climate upon plant growth in sand cultures. 91, 92, 102, 160

MAGISTAD, O. C., see GAUCH, H. G.

MAQUENNE, L., and DEMOUSSY, E. (1917). *C.R. Acad. Sci.* (*Paris*) **164** : 979. Influence de l'eau et des matières minérales sur la germination du pois. 52

MARAIS, J. S. (1922). *Soil Sci.* **13** : 355. The comparative agricultural value of insoluble mineral phosphates of aluminium, iron and calcium. 41

MARSH, R. P. (1923). *A.R. New Jersey agric. Exp. Stat.* 1922, **43** : 399. Comparison of iron from some organic compounds as an essential constituent of nutrient media for plants. 105, 108

MARCH, R. P. (1924a). *A.R. New Jersey agric. Exp. Stat.* 1923, **44** : 247. Ferric glycerophosphate and soluble ferric phosphate as source of iron for soya bean plants in solution cultures. 108

MARSH, R. P. (1924b). *A.R. New Jersey agric. Exp. Stat.* 1923, **44** : 252. Iron solubility tests in culture solutions at different pH values. 108

MARSH, R. P. (1935). *Science* **82** : 256. A modified culture jar. 124

MARSH, R. P. (1942). *Soil Sci.* **53** : 75. Comparative study of the calcium-boron metabolism of representative dicots and monocots. 112

MARSH, R. P., and SHIVE, J. W. (1925). *Bot. Gaz.* **79** : 1. Adjustment of iron supply to requirements of soya bean in solution culture. 13, 108

MARTIN, J. C., see HOAGLAND, D. R.

MARTIN, J. P. (1935). *Hawai. Plant. Rec.* **39** : 79. Studies on the growth of sugar cane in nutrient solutions. 25, 50, 103, 131

MARTIN, R. P., see SCOTT-RUSSELL, R.

MARTIN, W. C., see SHIVE, J. W.

MASKELL, E. J. (private communications). 124, 143, 153

MAYER (1901), given by PFEFFER (1910). 31

MAYNE, W. W. (1940). *Planters' Chron.* **35** : 327 and in slightly abridged form in *Trop. Agriculture, Trin.*, **18** : 13. Investigations on the nutrition of *Coffea arabica* L. 143

MAZÉ, P. (1900). *Ann. Inst. Pasteur.* **14** : 26. L'influence de l'azote nutrique et de l'azote ammoniacal sur le développement du mais. 3, 93, 95, T19A, 108

MAZÉ, P. (1914). *Ann. Inst. Pasteur.* **28** : 21. Recherche de physiologie végétale. Influence respective des éléments de la solution minerale sur le développement du mais. 3, 22, 93, 95, 108, 111

MAZÉ, P. (1915). *C.R. Acad. Sci.* (*Paris*), **160** : 211. Détermination des éléments minéraux rares nécessaires au développement du mais. 3, 11, 50, T19A, 108, 111, 114

MAZÉ, P. (1919). *Ann. Inst. Pasteur.* **33** : 139. Recherche d'une solution purément minérale capable d'assurer l'évolution complète du mais culturé à l'abri des microbes. 3, 67

MCCALL, A. G. (1916a). *Soil Sci.* **2** : 207. Physiological balance of nutrient solutions for plants in sand culture. 5, 6, 7, T4, 28, 83, 89, 90, T19A, 118, 137, 151, 154, 166

McCall, A. G. (1916b). *Ohio J. Sci.* **16** : 101. A method for the renewal of plant nutrients in sand cultures. 5, 6, 7, 89, 90, 118, 151

McCall, A. G. (1923). *J. Amer. Soc. Agron.* **15** : 290. The influence of acidity itself on plant growth without regard to other factors. 155

McCall, A. G., and Haag, J. R. (1921). *Soil Sci.* **12** : 69. The relation of the hydrogen ion concentration of nutrient solutions to growth and chlorosis of wheat plants. 99

McCall, A. G., and Richards, P. E. (1918). *J. Amer. Soc. Agron.* **10** : 127. Mineral food requirements of the wheat plant at different stages of its development. 5

McCalla, T. M., see Albrecht, W. A.

McCool, M. M. (1934). *Contr. Boyce Thompson Inst.* **6** : 147. Effect of various factors on the soluble manganese in soils. 159

McDermott, R. Given by Streets *et al.* (1946). 153

McEvoy, E. T. (1946). *Sci. Agric.* **26** : 640. Response of Burley tobacco varieties to ionic forms of nitrogen. 95, 96

McGeorge, W. T., and Breazeale, J. F. (1932). *Tech. Bull. Ariz. agric. Exp. Stat.* **40**. Studies on iron, aluminium and organic phosphates and phosphate fixation in calcareous soils. 41

McGrath, D., see Albert, A.

McHargue, J. S. (1922). *J. Amer. chem. Soc.* **44** : 1592. The rôle of manganese in plants. 3

McHargue, J. S. (1923a). *Soil Sci.* **16** : 359. Convenient supports for plants in pot or water cultures. 16, 17

McHargue, J. S. (1923b). *J. agric. Res.* **24** : 781. Effect of different concentrations of manganese sulphate on the growth of plants in acid and neutral soils and the necessity of manganese as a plant nutrient. 31, 32, 179

McHargue, J. S. (1923c). *J. agric. Res.* **26** : 231. Common earthenware jars as a source of error in pot experiments. 21

McHargue, J. S., and Calfee, R. K. (1932a). *Plant Physiol.* **7** : 161. Effect of boron on the growth of lettuce. 21, 23, 31

McHargue, J. S., and Calfee, R. K. (1932b). *Plant Physiol.* **7** : 697. Manganese essential for the growth of *Lemna major*. 21, 51, T19B

McHargue, J. S., and Calfee, R. K. (1933). *Plant Physiol.* **8** : 305. Further evidence that boron is essential for the growth of lettuce. 22, 41, 111, 114

McHargue, J. S., and Offut, E. B. (1940). *Industr. Engng Chem.* (*Anal.*) **12** : 157. Still for obtaining metal-free distilled water. 51, 52

McHargue, J. S., see Hageman, R. H.

McIlvaine, T. C., see Salter, R. M.

McLean, F. T., and Gilbert, B. E. (1926). *Soil Sci.* **24** : 163. The relative aluminium tolerance of crop plants. 10, 48, T19A, 104, 119

McMurtrey, J. E. Jr. (1929). *J. agric. Res.* **38** : 371. The effect of boron deficiency on the growth of tobacco plants in aerated and un-aerated solutions. 30, 50, 117, 151

McMurtrey, J. E. Jr. (1937). *J. agric. Res.* **55** : 475. Cross transfer of mineral nutrients in the tobacco plant. 25

McVeigh, I., see Burkholder, P. R.

Meagher, W. R., see Arnon, D. I.; Stout, P. R.

Mehlich, A., and Colwell, W. E. (1946). *Soil Sci.* **61** : 369. Absorption of calcium by peanuts from kaolin and bentonite at varying levels of calcium. 38, 39

Mehrlich, F. P. (1935). *Plant Physiol.* **10** : 169. A mechanism for controlled continuous flow of nutrient solutions. 28, 120, 125

Meites, L. Jr., see Lingane, J. J.

Mensching, J. E., see Collison, R. C.

Merkenschlager, F. (1927). "Tafeln zur vergleichenden Physiologie und Pathologie der Kulturpflanzen." Verlag Oscar Schlegel, Berlin. 104

Metz, O. (1930). *Arch. Mikrobiol.* **1** : 197. Über Wachstum und Farbstoffbildung einiger Pilze unter dem Einfluss von Eisen, Zink, und Kupfer. 73

Mevius, W. (1927). *Jb. wiss. Bot.* **66** : 183. Kalziumion und Wurzelwachstum. 104, 105

Mevius, W., and Dikussar, I. (1930). *Jb. wiss. Bot.* **73** : 633. Nitrite als Stickstoffquellen für höhere Pflanzen. Ein Beitrag zur Frage der Assimilation des Nitratstickstoffes. 97

Meyer, B. S. (1945). *Amer. J. Bot.* **32** : 523. Effects of deficiencies of certain mineral elements on the development of *Taraxacum kok-saghyz*. 29, 131, 151, 152

Meyer, B. S. (1946). *Amer. J. Bot.* **33** : 204. Effect of various concentrations of boron on the development of *Taraxacum kok-saghyz* in sand culture. 5, 29, 112, 131, 152

Miles, R. O. (1947). *Bull. Jealotts Hill Res. Stat.* **2** (Rev.). The culture of plants in sand and in aggregate. 127, 144

Millar, C. E., see Cook, R. L.

Millener, L. H. (private communication). 108

Miller, E. C. (1938). "Plant Physiology." 2nd Edit. McGraw Hill, New York. 89

Miller, L. P. (1928). *Amer. Fert.* **68** : 21. Manganese deficiency in sand culture. 20, 30, 50, 67

Miller, N. H. J. (1905). *J. agric. Sci.* **1** : 280. The amounts of nitrogen as ammonia and as nitric acid and of chlorine in the rainwater collected at Rothamsted. 42, 43

Miller, N. H. J., see Hutchinson, H. B.

Millikan, C. R. (private communications). 16, 135

Millikan, C. R. (1942). *J. Dep. agric. Vict.* **8** : 33. Symptoms of zinc deficiency in wheat and flax. 11, 15, 17, 22, 23, 54, 75, 151

MILLIKAN, C. R. (1944). *Proc. roy. Soc. Vict.* **56** : 113. Symptoms of copper deficiency in flax. 11, 15, 17, 22, 23, 54, 55, 75, 151

MILLIKAN, C. R. (1947a.) *J. Aust. Inst. agric. Sci.* **13** : 64. Zinc toxicity in flax grown in a wire netting " bird cage." 145

MILLIKAN, C. R. (1947b). *J. Aust. Inst. agric. Sci.* **13** : 180. Effect of molybdenum on the severity of toxicity symptoms in flax induced by an excess of either manganese, zinc, copper, nickel or cobalt in the nutrient solution. 111

MINARIK, C. E., and SHIVE, J. W. (1939). *Amer. J. Bot.* **26** : 827. The effect of boron in the substrate on calcium accumulation of soya bean plants. 31, 111

MITCHELL, H. L. (1939). *Black Rock Forestry Bull.* No. **9**. Growth and nutrition of *Pinus strobus* seedlings in cultures with varying N, P, K and Ca. 5, 21, 29, T19B, 119, 143, 145

MITCHELL, J. W., see NIGHTINGALE, G. T.

MITCHELL, K. J. (1950). *Nature* **166** : 35. High light intensities for experiments in crop physiology. 162

MITCHELL, R. L., and SCOTT, R. O. (1947). *J. Soc. chem. Ind. Lond.* **66** : 330. Concentration methods in spectrographic analysis II. Recovery of trace constituents in plant materials and soil extracts by mixed organic reagents. 79, 81

MITCHELL, R. L., and SCOTT, R. O. (1948). *Spectrochimica Acta* **3** : 367. Applications of chemical concentration by organic reagents to spectrographic analysis. 64, 79, 81

MITCHELL, R. L., see SCOTT, R. O.

MOCKERIDGE, F. A. (1924). *Ann. Bot. Lond.* **38** : 723. Auximones I. The formation of plant growth promoting substances by micro-organisms. 115

MOELLER, T. (1943a). *Industr. Engng Chem.* (*Anal.*) **15** : 270. The chemistry of indium. A colorimetric method for the estimation of small amounts of indium. 80

MOELLER, T. (1943b). *Industr. Engng Chem.* (*Anal.*) Extraction and colorimetric estimation of certain metals as derivatives of 8-hydroxyquinoline. 80

MOINAT, A. D. (1943). *Plant Physiol.* **18** : 517. Nutritional relations of boron and indolylacetic acid in head lettuce. 111

MOORE, W. J., see BEAUMONT, A. B.

MORRIS, S., and CRIST, J. W. (1927). *Tech. Bull. Mich. agric. Exp. Stat.* **77**. The influence of reaction of culture medium on growth of strawberry plants. 98

MUCKENHIRN, R. J. (1936). *J. Amer. Soc. Agron.* **28** : 824. Response of plants to boron, copper and manganese. 22, 30, T7, 41, 114

MULDER, E. G. (1939a). *Arch. Mikrobiol.* **10** : 72. Ueber die Bedeutung des Kupfers für das Wachstum von Mikroorganismen und über eine mikrobiologische Methode zur Bestimmung des pflanzenverfügbaren Bodenkupfers. 65, 73

MULDER, E. G. (1939b). *Antonie van Leeuwenhoek* **6** : 99. On the use of microorganisms in measuring a deficiency of copper, magnesium and molybdenum in soils. 73, 154

MULDER, E. G. (1948a). *Plant and Soil* **1** : 94. Importance of molybdenum in the nitrogen metabolism of microorganisms and higher plants. 10, 22, 67, 71, 94, T19B, 111, 135, 151, 154

MULDER, E. G. (1948b). *Analyt. chim. Acta* **2** : 793. The microbiological estimation of copper, magnesium and molybdenum in soil and plant material. 68

MULLISON, E. G., and MULLISON, W. R. (1949). *Proc. Amer. Soc. hort. Sci.* **54** : 452. Vegetable varieties for the tropics. 157

MULLISON, W. R. (1941). *Plant Physiol.* **16** : 813. The effect on barley seedlings of some interrelations of cations and anions in a 3-salt nutrient solution. 167

MULLISON, W. R., and MULLISON, E. (1942). *Plant Physiol.* **17** : 632. Growth responses of barley seedlings in relation to potassium and sodium nutrition. 87, 92, 166, 167

MYERS, R. J., and EASTES, J. W. (1941). *Industr. Engng Chem.* **33** : 1203. Synthetic-resin ion exchangers in water purification. 59

MYERS, R. J., EASTES, J. W., and MYERS, F. J. (1941). *Industr. Engng Chem.* **33** : 697. Synthetic resins as ion-exchange adsorbents. 59

MYERS, R. J., EASTES, J. W., and URQUART, D. (1933). *Industr. Engng Chem.* **33** : 1270. Adsorption isotherms of synthetic resin ion-exchange adsorbents. 59

MYERS, R. J., see HARRISON, J. W. E.

NAFTEL, J. A. (1931). *J. Amer. Soc. Agron.* **23** : 142. The absorption of NH_4– and NO_3–N by various plants at different stages of growth. 93, 95, 139

NEWELL, J., see LAWRENCE, W. J. C.

NEWTON, W. (1923). *J. Amer. Soc. Agron.* **15** : 392. The nutrition of the potato plant with special reference to water culture. 154

NICHOLAS, D. J. D. (1946). *Nature* **157** : 696. The detection of manganese deficiency in plants by tissue tests using tetramethyldiaminodiphenylmethane. 62, 69

NICHOLAS, D. J. D., and FIELDING, A. (1948). *A.R. Long Ashton agric. hort. Res. Stat.* 1947, p. 126. The use of *Aspergillus niger* (M strain) in the bio-assay of magnesium, copper, zinc and molybdenum in soil. 80, 81

NICHOLAS, D. J. D., and FIELDING, A. H. (1950). *Nature* **166** : 342. Use of *Aspergillus niger* as a test organism for determining molybdenum available in soils to crop plants. 79

NICHOLAS, D. J. D., and FIELDING, A. H. (1951). *J. hort. Sci.* **26** : 125. The use of *Aspergillus niger* (M) for the determination of magnesium, zinc, copper and molybdenum available in soils to crop plants. 68, 79, 80, 82

NICOL, H. (1934). *Biol. Rev.* **9** : 383. The derivation of the nitrogen of crop plants, with special reference to associated growth. 150

NICOL, H. (1936). *Mon. Bull. agric. Sci. Pract. Rome*, No. 6, p. 201 and No. 7, p. 241. The utilisation of atmospheric nitrogen by mixed crops. 150

NIERECKER, R., and TREADWELL, W. D. (1946). *Helv. chim. Acta* **29** : 1472. From abstract in *Analyst* **72** : 214. Quantitative determination of tungsten, molybdenum and vanadium alone or in the presence of ferric iron with 8-hydroxyquinoline. 80

NIGHTINGALE, G. T. (1933). *Bot. Gaz.* **95** : 35. Effect of temperature on metabolism in tomato. 161

NIGHTINGALE, G. T. (1934). *Bot. Gaz.* **95** : 437. Ammonium and nitrate nutrition of dormant Delicious apple trees at 48° F. 9, 21, 93, T19B, 100, 119, 141, 143, 155

NIGHTINGALE, G. T. (1937). *Bot. Rev.* **3** : 85. The nitrogen nutrition of green plants. 93, 95, 96

NIGHTINGALE, G. T., ADDOMS, R. M., ROBBINS, W. R., and SCHERMERHORN, L. G. (1931). *Plant Physiol.* **6** : 605. Effects of calcium deficiency on nitrate absorption and on metabolism in tomato. 118

NIGHTINGALE, G. T., and BLAKE, M. A. (1934a). *Bull. New Jersey agric. Exp. Stat.* **566**. The effects of temperature on the growth and composition of Stayman and Baldwin apple trees. 118, 141, 153, 161

NIGHTINGALE, G. T., and BLAKE, M. A. (1934b). *Bull. New Jersey agric. Exp. Stat.* **567**. Effects of temperature on the growth and metabolism of Elberta peach trees with notes on the growth response of other varieties. 141, 143, 161

NIGHTINGALE, G. T., and FARNHAM, R. B. (1936). *Bot. Gaz.* **97** : 477. Effects of nutrient concentration on anatomy, metabolism and bud abscission of sweet pea. 91

NIGHTINGALE, G. T., and MITCHELL, J. W. (1934). *Plant Physiol.* **9** : 217. Effects of humidity on metabolism in tomato and apple. 160, 161

NIGHTINGALE, G. T., SCHERMERHORN, L. G., and ROBBINS, W. R. (1932). *Plant Physiol.* **7** : 565. Effcets of sulphur deficiency on metabolism in tomato. 118

NIGHTINGALE, G. T., see BLAKE, M. A.

NIKLEWSKI, B., KRAUSE, A., and LEMANEZYK, K. (1928). *Jb. wiss. Bot.* **69** : 101. The knowledge of the mechanism of the absorption of mineral constituents by the roots of plants. 98

NILSSON, P. E. (1950). *Ann. roy. agric. Coll. Sweden* **17** : 358. An improved device for aseptic cultivation of higher plants. 150

NOBBE, F. (1865). *Landw. VersSta.* **7** : 68. Die Züchtung der Landpflanzen im Wasser betreffend. 83, 119

NOBBE, F. (1868). *Landw. VersSta.* **10** : 1. Beiträge zur Pflanzenkultur in tropfbar flüssigen Wurzelmedien. 3, 119

NOBLE, E. L., see TARR, L. W.

NOGGLE, G. R., see WATSON, S. A.

NOREM, W. L. (1936). *Amer. J. Bot.* **23** : 545. Mineral nutrition and seasonal growth of *Ageratum* in sand cultures with auto-irrigation. 122

NOSTITZ, A. (1925). *Landw. VersSta.* **105** : 159. Zur Bedeutung der basisch austauschbaren Bodennährstoffe für die Pflanzen und über Einwirkung des Kalkes auf die absorbierenden Bodenkörper. 35, 38, 41, 96

OERTEL, A. C., and PRESCOTT, J. A. (1944). *Trans. roy. Soc. South Australia* **68** : 173. A spectrochemical examination of some ironstone gravels from Australian soils. 30

OERTEL, A. C., see PIPER, C. S.

OFFUT, E. B., see MCHARGUE, J. S.

OLSEN, C. (1935). *C.R. Lab. Carlsberg* **21** : 15. Iron absorption and chlorosis in green plants. 103, 105, 106

ONDRATSCHEK, K. (1941). *Arch. Mikrobiol.* **12** : 241. From *Biol. Abst. D.* **19** : 17386. Über den Mineralsalzbedarf heterotropher Flagellaten. 73

OVERSTREET, R., see JENNY, H. ; STOUT, P. R.

PADDICK, M. E. (1944). *Plant Physiol.* **19** : 704. A simple apparatus for sand culture work. 131

PAGAN, V., see HOPKINS, E. F.

PAGE, H. J., see GREENHILL, A. W.

PARDO, J. H. (1935). *Quart. Rev. Biol.* **10** : 1. Ammonium in the nutrition of higher green plants. 93, 94, 95, 96

PARKER, E. R., see CHAPMAN, H. D.

PARKER, F. W. (1927). *Soil Sci.* **24** : 129. Soil phosphorus studies III. Plant growth and the absorption of phosphorus from culture solutions of different phosphorus concentrations. 11, 16, 21, 87, 106, 117, 139

PARKER, F. W., and PIERRE, W. H. (1928). *Soil Sci.* **25** : 337. The relation between the concentration of mineral elements in the culture media and the absorption and utilisation of the elements by plants. 12, 14, 18, 86, 87, 117, 139

PARKER, H. C., see CURTIN, L. P.

PARKER, M. W. (1946). *Soil Sci.* **62** : 109. Environmental factors and their control in plant experiments. 162

PARKER, M. W., and BORTHWICK, H. A. (1949). *Plant Physiol.* **24** : 345. Growth and composition of Biloxi soya beans in a controlled environment with radiation from different carbon arc sources. 162

PARKER, M. W., and BORTHWICK, H. A. (1950). *Plant Physiol.* **25** : 86. A modified circuit for Slimline fluorescent lamps for plant growth chambers. 162

PARKS, R. Q. (private communication). 162

PARTINGTON, J. R. (1933). " Textbook of inorganic chemistry." 4th Edit. MacMillan, London. 195

PEARSE, H. L. (1937). *A.R. East Malling Res. Stat.* 1936, p. 131. Apple trees in water culture. 12, 16, 106, 122, 131, 135, 141, 142, 165

PEARSE, H. L. (1940). *J. Pomol.* **17** : 344. Water culture studies with apple trees II. The seasonal absorption of nitrogen and potassium by Cox's Orange Pippin on Malling rootstocks Nos. IX and XII. 16, 110, 122, 141, 165

PEARSE, H. L. (1944). *Fmg S. Africa* **19** : 688. Iron and manganese in plant nutrition. 54, 108

PELTIER, G. L., and GOSS, R. W. (1924). *Res. Bull. Neb. agric. Exp. Stat.* **28**. Control equipment for the study of the relation of environment to disease. 161

PEMBER, F. R., see GILBERT, B. E. ; HARTWELL, B. L.

PEMBERTON, R., see WELLS, J. E.

PENAN, H., see CLIQUET, R.

PENTZ, E. I., see Young, E. G.

PEPKOWITZ, L. P., and SHIVE, J. W. (1944). *Soil Sci.* **57** : 143. The importance of oxygen in the nutrient substrate for plants. 133, 137

PERLMAN, D. (1945). *J. Bact.* **49** : 167. Some effects of metallic ions on the metabolism of *Aerobacter aerogenes*. 82

PETERSBURGSKY, A. V. (1945). *Trudy. S.-Kh. Akad. Timiriazeva* **30** : 21. From abstr. *Soils and Ferts.* **9** : 12. Adsorption by plants of cations from the solid phase. 36

PETERS, C. A., see JONES, L. H.

PFEFFER, W. (1905). " The physiology of plants." 2nd German Edit. 1900 3 vols. translated by A. J. Ewart, Clarendon Press, Oxford. T19A

PFEFFER, W. (1918). " Die Vegetationsversuche," Berlin. 2, 31, 32, 159

PIEKENBROCK, P., see BEAUMONT, A. B.

PIERRE, W. H., see PARKER, F. W.

PIPER, C. S. Waite Agricultural Institute (private communications). 11, 17, 22, 23, 52, 54, 55, 71, 76, 81, 135, 137, 164, 194

PIPER, C. S. (1940). *J. Aust. Inst. agric. Sci.* **6** : 112. Molybdenum as an essential element for plant growth. 17, 23, 54, 67, 71, 76

PIPER, C. S. (1941). *J. agric. Sci.* **31** : 448. Marsh spot of peas. A manganese deficiency disease. 11, 17, 23, 54, 70, 71, 81, 82, 110, 112, 113, 158

PIPER, C. S. (1942). *J. agric. Sci.* **32** : 143. Investigations on copper deficiency in plants. 11, 16, 17, 23, 24, 54, 55, 70, 71, 74, 75, 80, 81, 86, T19B, 110, 112, 113, 114, 133, 151, 158

PIPER, C. S. (1944). *Nature* **153** : 197. Manganese deficiency in oats. 22, 51, 52, 66, 76, 164

PIPER, C. S., and OERTEL, A. C. (1941). *Industr. Engng Chem.* (*Anal.*) **13** : 491. Silica and pyrex glass stills made with automatic constant pressure feeds. 51, 52, 54, 55, 56, 186

PIPER, C. S., see SAMUEL, G.

PIRSCHLE, K. (1929). *Planta* **9** : 84. Nitrat- und Ammonsalze als Stickstoffquellen für höhere Pflanzen bei konstanter Wasserstoffionen Konzentration. 9, 13, 124

PIRSCHLE, K. (1931a). *Planta* **14** : 583. Nitrat- und Ammonsalze als Stickstoffquellen für höhere Pflanzen bei konstanter Wasserstoffionen Konzentration. 9, 13, 96, 98

PIRSCHLE, K. (1931b). *Z. PflErnähr. Düng.* A **22** : 51. Nitrat- und Ammonsalze als Stickstoffquellen für höhere Pflanzen bei konstanter Wasserstoffionen Konzentration. 94, 96

POLLARD, A. G., and SMITH, C. W. R. (1943). *J. roy. hort. Soc.* **68** : 201. Soil studies at Wisley 1. Observations on pot culture. 20

POLLARD, A. G., and SMITH, C. W. R. (1943). *J. roy. hort. Soc.* **68** : 239. Soil studies at Wisley 2. Further observations on potted soils. 20

POLLARD, A. G., SMITH, C. W. R., and ROWE, L. R. (1943). *J. roy. hort. Soc.* **68** : 271. Soil studies at Wisley 3. The flower pot, the soil and the plant. 20

POLSTORF, see WIEGMAN

PONTOVICH, W. E., see TAUSSON, W. O.

PORTSMOUTH, G. B. (1947, unpublished) 42

PORTSMOUTH, G. B. (1949). *Ann. Bot. Lond.* **13** n.s. : 113. The effect of manganese on carbon assimilation in the potato plant as determined by a modified half-leaf method. 169

POSNETTE, A. F., see GREENWOOD, M.

POUGET, I., and CHOUCHAK, D. (1912). *C.R. Acad. Sci.* (*Paris*) **155** : 303. Sur la loi du minimum. 124

POWERS, W. L., see AHI, S. M. ; LEWIS, J. C.

PRESCOTT, J. A., see OERTEL, A. C.

PRESLEY, J. T., see CAMPBELL, W. A.

PRESTON, R. ST. J. (1947). " Bituminous coatings for protection of iron and steel against corrosion." *D.S.I.R. Chem. Res. Lab. Spec. Rep.* No. **5**. 18, 47

PRIANISHNIKOV, D. N., and DOMONTOVITCH, M. K. (1926). *Soil Sci.* **21** : 327. The problem of a proper nutrient medium. 40, 156

PROKOVIEV, A. A., see TAUSSON, W. O.

PROVASOLI, L., see HUTNER, S. H.

PUCHER, G. W., LEAVENWORTH, C. S., GINTER, W. D., and VICKERY, H. B. (1947). *Plant Physiol.* **22** : 205. Studies in the metabolism of plants. The effect upon the composition of *Bryophyllum calycinum*, of the form in which N is supplied. 101

PURVIS, E. R., see EVANS, H. J.

QUASTEL, J. H., see AUDUS, L. J.

RADER, L. F., and HILL, W. L. (1938). *J. agric. Res.* **57** : 901. Determination and occurrence of boron in natural phosphates, superphosphates and defluorinated phosphate rocks. 64

RAINES, M. A. (1937). *Amer. J. Bot.* **24** : 185. Wick culture of seedlings with different rates of water flow. 122

RAINES, M. A. (1939a). *Science* **89** : 205. A modification of the set-up for wick culture. 122

RAINES, M. A. (1939b). *Plant Physiol.* **14** : 829. A metering pump for small volumes of solution. 127

RALEIGH, G. J. (1939). *Plant Physiol.* **14** : 823. Evidence for the essentiality of silicon for the growth of the beet plant. 4, 17, 18, 22, 82

RAMIG, R. E., and VANDECAVEYE, S. C. (1950). *Plant Physiol.* **25** : 617. A study of certain nutrient levels for raspberries grown in water culture. 12, 16, 137, 150

RAMIREZ-SILVA, F. J., see HOPKINS, E. F.

RANKIN, E. J., see TOTTINGHAM, W. E.

RAPP (1928) given by ROBERG (1931). 54

RAULIN, J. (1863). *C.R. Acad. Sci. (Paris)* **57** : 228. Études chimiques sur la végétation des Mucodinées particulièrement de l'*Ascophora nigrans*. 3

RAY, T. W. (1940). *J. Lab. clin. Med.* **25** : 745. A rapid means of obtaining manganese-free iron. 82

RAYNER, D. S., see CHAPMAN, H. D.

READ, F. M. (Victoria, Australia) (private communications). 15, 48, T19B, 141

REDDINGTON (1940). Given by EVANS (1946). 47

REED, H. S. (1942). " A short history of the plant sciences." Chronica Botanica, New York. 2

REED, H. S., and HAAS, A. R. C. (1923a). *J. agric. Res.* **24** : 801. Growth and composition of orange trees in sand and soil cultures. 5, 8, 18, T4, 27, 28, 143

REED, H. S., and HAAS, A. R. C. (1923b). *Tech. Pap. Calif. agric. Exp. Stat.* **4**. Effect of sodium chloride and calcium chloride upon growth and composition of young orange trees. 143

REED, H. S., and HAAS, A. R. C. (1924a). *J. agric. Res.* **28** : 277. Some relations between the growth and composition of young orange trees and the concentration of the nutrient solution employed. 10, 89, 90, T19B, 143

REED, H. S., and HAAS, A. R. C. (1924b). *Amer. J. Bot.* **11** : 78. The effect of hydroxyl ion concentration on the growth of walnut roots. 99

REED, H. S., and HAAS, A. R. C. (1924c). *Bot. Gaz.* **77** : 290. Iron supply in a nutrient medium. 103

REED, H. S., see HAAS, A. R. C.

REEVE, E., and SHIVE, J. W. (1944). *Soil Sci.* **57** : 1. Potassium-boron, and calcium-boron relationships in plant nutrition. 21, 112, 113, 120, 143, 155

RICHARDS, F. J. (1932). *Ann. Bot. Lond.* **46** : 367. Physiological studies in plant nutrition III. Further studies of the effect of potash deficiency on the rate of respiration in leaves of barley. 21, 40, 92

RICHARDS, F. J. (1938). *Ann. Bot. Lond.* **2** n.s. : 491. Physiological studies in plant nutrition. VIII. The relation of respiration rate to the carbohydrate and nitrogen metabolism of the barley leaf as determined by phosphorus and potassium supply. 21, 40

RICHARDS, F. J. (1941). *Ann. Bot. Lond.* **5** n.s. : 263. Physiological studies in plant nutrition. XI. The effect on growth of rubidium with low potassium supply, and modification of this effect by other nutrients. Part I. The effect on total dry weight. 93

RICHARDS, F. J. (1944). *Ann. Rev. Biochem.* **13** : 611. Mineral nutrition of plants. 167

RICHARDS, F. J. (1945). Report No. **7859**, to the Mineral Deficiencies Conference of the Agricultural Research Council, " Report on experiments on chlorosis carried out in 1945." 24, 25, T19B, 119

RICHARDS, F. J. (1946). Report No. **8463**, to the Mineral Deficiencies Conference of the Agricultural Research Council " Report on the chlorosis experiments of 1945." 24

RICHARDS, F. J., and SHIH, SHENG-HAN (1940a). *Ann. Bot. Lond.* **4** n.s. : 165. Physiological studies in plant nutrition. X. Water content of barley leaves as determined by the interaction of potassium with certain other nutrient elements. Part I. The relationship between water content and nutrient composition. 93

RICHARDS, F. J., and SHIH, SHENG-HAN (1940b). *Ann. Bot. Lond.* **4** n.s. : 403. Physiological studies in plant nutrition. X. Water content of barley leaves as determined by the interaction of potassium with certain other nutrient elements. Part 2. The relationship between water content and composition of the leaves. 93

RICHARDS, F. J., and TEMPLEMAN, W. G. (1936). *Ann. Bot. Lond.* **50** : 367. Physiological studies in plant nutrition. IV. Nitrogen metabolism in relation to nutrient deficiency and age in leaves of barley. 21, 40, 87, 92, 149

RICHARDS, F. J., see GREGORY, F. G.

RICHARDS, L. A., and BLOOD, H. L. (1934). *J. agric. Res.* **49** : 115. Some improvements in auto-irrigator apparatus. 124

RICHARDS, P. E., see MCCALL, A. G.

RICHTER given by SCHROPP (1934). 108

ROBBINS, W. R. (1937). *Plant Physiol.* **12** : 26. Relation of nutrient concentration to growth of the tomato and to the incidence of blossom-end rot of the fruit. T4, 91

ROBBINS, W. R. (1946). *Soil Sci.* **62** : 3. Growing plants in sand cultures for experimental purposes. 5, 9, 16, 21, T4, 31, 32, 86, 88, 90, 92, 94, 96, T19B, 98, 100, 101, 118, 120, 152, 153, 156, 159, 160

ROBBINS, W. R., see HAYNES, J. L. ; KOLODNEY, L. ; NIGHTINGALE, G. T. ; SHIVE, J. W. ; TIEDJENS, V. A. ; WADLEIGH, C. H.

ROBERG, M. (1928). *Zbl. Bakt.* (II) **74** : 333. Über die Wirkung von Eisen, Zink und Kupfersalzen auf *Aspergillen*. 20, 23, 52, 54, 70, 71, 72, 74

ROBERG, M. (1931). *Zbl. Bakt.* (II) **84** : 196. Weitere Untersuchungen über die Bedeutung des Zinks für *Aspergillus niger*. 52, 54, 55, 67, 68, 73

ROBERG, M. (1932). *Jb. wiss. Bot.* **76** : 311. Ein Beitrag zur Stoffswechsel-Physiologie der Grünalgen. II. Über die Wirkung von Eisen, Zink und Kupfersalzen. 54, 71, 72

ROGERS, L. H., see GADDUM, L. W.

DE ROPP, R. S. (1946). *Science* **104** : 371. Apparatus for the prolonged sterile culture of whole plants. 150

ROTHE, given by FALES, H. A. (1928), p. 273, HILLEBRAND, W. E., and LUNDELL, G. E. F. (1929), p. 106. 81

ROWE, E. A. (1936). *Ann. Bot. Lond.* **50** : 735. A study of ' heart rot ' of young sugar beet plants grown in culture solutions. 112, 151

ROWE, L. R., see POLLARD, A. G.

ROY, W. R., and GARDNER, F. E. (1946). *Proc. Amer. Soc. hort. Sci.* **47** : 107. Seasonal absorption of nutrient ions by orange trees in sand culture. 8, 21, 118, 143

RUBENS, E. J., see DEAN, L. A.

RUCK, H. C. (1951, private communication), see BOLAS and RUCK (1949). 17, 153

RUSTAN, H. G., see CROWTHER, C.

RUTTER, A. J., see BLACKMAN, G. E.

SACHS, J. VON. 3, 16, 133, 151, 169

SACHS, J. VON (1860). *Landw. VersSta.* **2** : 219. Berichte über die physiologische Thätigheit an der Versuchsstation in Tharandt. IV. Vegetationsversuche mit Ausschluss des Bodens über die Nährstoffe und sonstigen Ernährungsbedingungen von Mais, Bohnen und anderen Pflanzen. 2, 11, 93, T19A, 103

SAEGER, A. C. (1937). *Amer. J. Bot.* **24** : 640. The concentration of copper in the nutrient solutions for *Spirodella polyrhiza*. 71, 82, 154

SAKAMURA, T. (1936). *J. Fac. Sci. Hokkaido imp. Univ. Ser. V Botany* **4** : 99. Über einige für die Kultur von Aspergillen notwendige Schwermetalle und das Befreiungsverfahren der Nährlösung von ihren Spuren. 73, 74

SALM-HORSTMAR, F. (1849). *J. prakt. Chem.* **1**. Versuche über die nothwendigen Aschenbestandtheile einer Pflanzen-Species. 2, 26, 102

SALM-HORSTMAR, F. (1851). *Ann. Chim.* (Phys.) **32** : 461. Recherches sur la nutrition de l'avoine particulièrement en ce qui concerne les matières qui sont nécessaires à cette nutrition. 2, 3, 6, 7, 20, 26, 31, 41, 70, 102, 114

SALTER, R. M., and MCILVAINE, T. C. (1920). *J. agric. Res.* **19** : 73. Effect of reaction of solution on germination of seed and on growth of seedlings. 98, 99, 151

SAMUEL, G., and PIPER, C. S. (1929). *Ann. appl. Biol.* **16** : 493. Manganese as an essential element for plant growth. 11, 22, 66, 67, 82

SANDELL, E. B. (1944). " Colorimetric determination of traces of metals." Interscience, New York. 80, 81, 195

SANDELL, E. B. (1947). *Anal. Chem.* **19** : 63. Determination of gallium in silicate rocks. 81

DE SAUSSURE, TH. (1804). " Recherches chimiques sur la végétation." Paris. 2

SAYRE, J. D., see CONVERSE, C. D.

SCHAFER, J. Jr., see SCHROEDER, W. T.

SCHAPPELLE, N. A. (1942). *J. agric. Univ. Puerto Rico* **26** : 63. The influence of pH and of certain minor elements on the growth of pineapples in water cultures. 12, 99, 101, 112, 113, 152

SCHARRER, K., and GOTSCHALL, R. (1935). *Z. PflErnähr. Düng.* **39** : 178. Zur Methodik der Bestimmung kelinster Mengen Borsäure. 196

SCHARRER, K., and SCHROPP, W. (1933). *Z. PflErnähr. Düng.* A **32** : 184. Sand- und Wasserkulturversuch über die Wirkung des Kupfer-ions. T7, 155

SCHARRER, K., and SCHROPP, W. (1936). *Z. PflErnähr. Düng.* **45** : 83. Über die Wirkung des Aluminiumions auf das Pflanzenwachstum. 155

SCHARRER, K., see SCHROPP, W.

SCHATZ, A., see HUTNER, S. H.

SCHERMERHORN, L. G., see NIGHTINGALE, G. T.

SCHIMPER, A. F. Z. (1890). *Flora* **73** : 207. Zur Frage der Assimilation der Mineralsalze durch die grünen Pflanzen. T19A, 104

SCHLENKER, F. S. (1940). *Amer. J. Bot.* **27** : 525. Plant growth in culture solutions and availability of ions adsorbed on Permutit and aniline black. 34, 36

SCHLENKER, F. S. (1942). *Soil Sci.* **54** : 247. Availability of adsorbed ions to plants growing in quartz sand substrate. 36

SCHLOESSING, TH. (fils) (1899). *Ann. Sci. agron. Paris* **1** : 316. Étude sur l'acide phosphorique dissous par les eaux du sol. 3, 120, 121

SCHMIDT, D. (1921). *A.R. New Jersey agric. Exp. Stat.* 1920, p. 333. Relation of seed weight to the rôle of growth and size of crimson clover plants. 164

SCHMIDT, D. (1923). *A.R. New Jersey agric. Exp. Stat.* 1922, p. 388. A study of plant growth in relation to the weight of seed. 164

SCHOOREL, A. F., see DE HAAN, I.

SCHOLZ, W. (1936). *Z. PflErnähr. Düng.* **43** : 1. Die Reinigung von Hohenbockaer Quarzsand zwecks Durchführung von Nährstoffmangelversuchen. 31, 32

SCHREINER, O., and SKINNER, J. J. (1910). *Bot. Gaz.* **50** : 1. Ratio of phosphate, nitrate, and potassium on absorption and growth. 3, 10, 14, 17, T19A, 151, 167

SCHREVEN, D. A. VAN (1934a). *Tijdschr. PlZiekt.* **40** : 97. Uitwendige en inwendige symptomen van boriumgebrek bij tabak. 22, 29, 31

SCHREVEN, D. A. VAN (1934b). *Tijdschr. PlZiekt.* **40** : 226. Kalkgebrek als oorzaak van mergnecrose bij aardappelknollen. 15, 29, 31, 40, 41

SCHREVEN, D. A. VAN (1936a). *Phytopathology* **26** : 1106. Copper deficiency in higher plants. 17, 22, 54, 70, 151

SCHREVEN, D. A. VAN (1936b). *Meded. Inst. Zuckerbiet Bergen o.Z.* **6** (part 2) : 37. Kopergebrek bij de Zuckerbiet. 70, 151

SCHREVEN, D. A. VAN (1939). *Meded. Landb-Hoogesch. Wageningen* **43** (part 1). De gezondheidstoestand van de aardappelplant onder den invloed van twaalf elementen. 31, 50, 112

SCHROEDER, R. A., see WITTWER, S. H.

SCHROEDER, W. T., DAVIS, J. F., and SCHAFER, J. Jr. (1946). *J. Amer. Soc. Agron.* **38** : 754. Deionised water not a suitable substitute for distilled water in boron studies. 50, 57, 59

SCHROPP, W. (1932). *Fortschr. Landw.* **7** : 129. Über die Regelung der Standortsverhältnisse bei Vegetationsversuchen in Gefässen. 147

SCHROPP, W. (1934). *Z. PflErnähr. Düng.* A **33** : 38. Zur Eisenversorgung der Pflanzen bei Wasserkulturversuchen. 108

SCHROPP, W. (1936). *Z. PflErnähr. Düng.* **42** : 35. Zur Eisenversorgung der Pflanzen bei Wasserkulturversuchen. 106

SCHROPP, W. (1938). *Z. PflErnähr. Düng.* **54/55** (n.s. **9/10**) : 653. Zur Eisenversorgung der Pflanzen bei Wasserkulturversuchen. 107

SCHROPP, W. (1941). *Tropenpflanzer* Nos. **11-12**. Über die Wirkung des Bors auf subtropische und tropische Kulturpflanzen. 157

SCHROPP, W., and ARENZ, B. (1938). *Forschungsdienst,* **6** : 564. Über die Wirkung des Bors auf das Wachstum einiger Öl- und Gespinstpflanzen. 17, 22

SCHROPP, W., and ARENZ, B. (1940). *Z. PflErnähr. Düng.* **61** (n.s. **16**) : 169. Versuche mit Bor zu einigen Gramineen. 17, 19, 52

SCHROPP, W., and SCHARRER, K. (1933). *Jb. wiss. Bot.* **78** : 544. Wasserkulturversuche mit den A-Z Lösungen nach Hoagland. 108, 109

SCHROPP, W., and SOUKUP, H. (1935). *Landw. VersSta.* **122**, (parts 4, 5 and 6). Die Mangelversuche in Form der Wasserkultur. 105

SCHROPP, W., and ZOLLER, E. (1934). *Landw. VersSta.* **118**, (parts 1 and 2). Die Mangelversuche in Form der Wasserkultur. 105

SCHROPP, W., see SCHARRER, K.

SCHUFFELEN, A. C., and LOOSJES, R. (1942). *Proc. kon. Ned. Akad. Wet. Amst.* **45** : 726. Abstr. in *Soils and Ferts.* **9** : 301. Importance of the growth medium for the absorption of cations by plants. 84

SCOTT, D. H., see CULLINAN, F. P.

SCOTT, R. O., and MITCHELL, R. L. (1943). *J. Soc. chem. Ind. Lond.* **62** : 5. Concentration methods in spectrographic analysis 1. Recovery of cobalt, nickel, molybdenum, copper and zinc from plant materials and soil extracts by 8 hydroxyquinoline. 78

SCOTT, R. A., see MITCHELL, R. L.

SCOTT-RUSSELL, R., and MARTIN, R. P. (1949). *Nature* **163** : 61. Use of radioactive isotopes in plant nutritional studies. 116

SCOTT-RUSSELL, R., and MARTIN, R. P. (1950a). *J. exp. Bot.* **1** : 133. Studies with radioactive tracers on plant nutrition. I. Problems in the use of radioactive tracers. 117

SCOTT-RUSSELL, R., and MARTIN, R. P. (1950b). *J. exp. Bot.* **1** : 141. II. The estimation of radioactive tracers. 117

SEBOR, J., see STOKLASA, J.

SEN, P. K., see GREGORY, F. G.

SEUFT, E., see STOKLASA, J.

SHANTZ, H. L., see BRIGGS, L. J.

SHAPTER, R. F., see TRUMBLE, H. C.

SHEPARDSON, W. B., see JONES, L. H.

SHERMAN, G. D., see HAGEMAN, R. H.

SHERMAN, M. S., see HORNER, C. K.

SHIH, SHENG-HAN, see RICHARDS, F. J.

SHIRLEY, H. L. (1929). *Contr. Boyce Thompson Inst.* **2** : 159. The influence of light intensity and light quality upon the growth of plants. 159

SHIVE, J. W. (1915a). *Physiol. Res.* **1** : 327. A study of physiological balance in nutrient media. 3, 10, 14, 88, 89, T19A, 151, 167

SHIVE, J. W. (1915b). *Amer. J. Bot.* **2** : 157. A three-salt nutrient for plants. 3, 10, 14, 83, 88, 89, 165

SHIVE, J. W. (1918a). *Soil Sci.* **6** : 1. A comparative study of the salt requirements for young and mature buckwheat plants in sand cultures. 5, 6, 7, 14, T4, 28, 32, 83, 88, 89, 118, 137, 155

SHIVE, J. W. (1918b). *Bull. New Jersey agric. Exp. Stat.* **319**. A study of physiological balance for buckwheat grown in 3-salt solutions. 14, 89

SHIVE, J. W. (1920a). *Soil Sci.* **9** : 169. The influence of sand upon the concentration and reaction of a nutrient solution for plants. 28, 32

SHIVE, J. W. (1920b). *J. agric. Res.* **18** : 357. Relation of moisture in solid substrate to physiological salt balance for plants and to the relative plant-producing value of various salt proportions. 7, 28

SHIVE, J. W. (1923). *A.R. New Jersey agric. Exp. Stat.* 1922, p. 374. Effect of aeration and continuous solution renewal on the salt requirements for soya beans in water culture. 127, 135

SHIVE, J. W. (1938). *Bull. New Jersey agric. Exp. Stat.* **603**. The adequacy of the boron and manganese content of natural nitrate of soda to support plant growth in sand cultures. 5, 9, 30, 65, 67, 68

SHIVE, J. W. (1940a). *Sci. Mon.* **51** : 233. Sketch of the development of the water culture method of growing plants. 2

SHIVE, J. W. (1940b). *Circ. New Jersey agric. Exp. Stat.* **399**. Vitamin B_1. Its use as a growth regulating substance for green plants. 115, 116

SHIVE, J. W. (1941). *Soil Sci.* **51** : 445. The balance of ions and oxygen tension in the nutrient. 133, 137

Shive, J. W., and Martin, W. H. (1918a). *J. agric. Res.* **14** : 151. A comparative study of salt requirements for young and for mature buckwheat plants in solution cultures. 10, 156, 166

Shive, J. W., and Martin, W. H. (1918b). *Amer. J. Bot.* **5** : 186. A comparison of salt requirements for young and for mature buckwheat plants in water cultures and sand cultures. 10, 155

Shive, J. W., and Robbins, W. R. (1942). *Bull. New Jersey agric. Exp. Stat.* **636**. Methods of growing plants in solution and sand cultures. 5, 9, 13, 16, 21, 22, 85, 92, 96, T19B, 98, 101, 108, 118, 119, 120, 124, 135, 151, 152, 159

Shive, J. W., and Stahl, A. L. (1927). *Bot. Gaz.* **84** : 317. Constant rates of solution renewal for plants in water cultures. 102, 120, 123, 124

Shive, J. W., see Allison, R. V.; Arrington, L. B.; Barnette, R. M.; Beckenbach, J. R.; Clark, H. E.; Davidson, O. W.; Gilbert, B. E.; Jones, L. H.; Marsh, R. P.; Minarik, C. E.; Pepkowitz, L. P.; Reeve, E.; Somers, J. J.; Spencer, E. L.; Stahl, A. L.

Sideris, C. P., and Krauss, B. H. (1937). *Growth* Sept. 1937, p. 204. The growth of pineapple shoots in complete water cultures with either ammonia or nitrate salts. 96

Sideris, C. P., Krauss, B. H., and Young, H. Y. (1938). *Plant Physiol.* **13** : 489. Assimilation of ammonium and nitrate by pineapple plants grown in nutrient solutions and their effects on nitrogenous and carbohydrate constituents. 11, 109

Sideris, C. P., and Young, H. Y. (1946). *Plant Physiol.* **21** : 247. Effects of nitrogen on growth and ash constituents of *Ananas comosus* (L) Merr. 10, 12, 111, 113, 114

Sideris, C. P., and Young, H. Y. (1949). *Plant Physiol.* **24** : 416. Growth and chemical composition of *Ananas comosus* (L) Merr. in solution cultures with different iron-manganese ratios. 86

Sideris, C. P., Young, H. Y., and Krauss, B. H. (1943). *Plant Physiol.* **18** : 608. Effects of iron on the growth and ash constituents of *Ananas comosus* (L) Merr. 12, 67, 95, T19B, 111, 113, 152.

Silberstein, L., see Bertrand, G.

Sipos, F., see Arnon, D. I.

Skinner, C. E., see Jones, C. D.

Skinner, J. J., see Schreiner, O.

Skok, J. (1941). *Bot. Gaz.* **103** : 200. Effect of boron on growth and development of the radish. 159

Small, J. (1946). "pH and plants." Bailliere, Tindall and Cox, London. 102

Smith, C. W. R., see Pollard, A. G.

Smith, M. E. (1943). *Aust. For.* **7** : 22. Micronutrients essential for the growth of *Pinus radiata*. 11, 12, 18, 22, 51, 143

Smith, M. E., and Bayliss, N. S. (1942). *Plant Physiol.* **17** : 303. The necessity of zinc for *Pinus radiata*. 10, 15, 17, 22, 23, 54, 80, 135, 143, 152, 164

Smith, S. N. (1934). *J. Amer. Soc. Agron.* **26** : 785. Response of inbred lines and crosses in maize to variations of nitrogen and phosphorus supplied as nutrients. 165

Snyder, W. C., see Hoagland, D. R.

Solberg, P. (1935). *Landw. Jb.* **81** : 891. Untersuchungen über die Wurzelausscheidenungen verschiedener Pflanzen bei ihrer Kultur ohne und mit Zusatz von Nährsalzen. 101

Somers, J. J., and Shive, J. W. (1942). *Plant Physiol.* **17** : 582. The iron-manganese relation in plant nutrition. 108

Sommer, A. L. (1926). *Univ. Calif. Publ. agric. Sci.* **5** : 57. Studies concerning the essential nature of aluminium and silicon for plant growth. 4, 22, T19B, 111, 164

Sommer, A. L. (1928). *Plant Physiol.* **3** : 217. Further evidence of the essential nature of zinc for the growth of higher green plants. T19B, 109

Sommer, A. L. (1931). *Plant Physiol.* **6** : 339. Copper as an essential element for plant growth. 4, 10, 17, 22, 52, 54, 70, 109

Sommer, A. L. (1936). *J. agric. Res.* **52** : 133. The relationship of the phosphate concentration of solution cultures to the type and size of root systems and the time of maturity of certain plants. 13, 18, 87, 117, 133, 144

Sommer, A. L., and Lipman, C. B. (1926). *Plant Physiol.* **1** : 231. Evidence of the indispensable nature of zinc and boron for higher green plants. 3, 22, 23, 52, 54

Sommer, A. L., and Sorokin, H. (1928). *Plant Physiol.* **3** : 237. Effects of the absence of boron and some other essential elements on the cell and tissue structure of the root tips of *Pisum sativum*. 70, 109

Sorokin, H., see Sommer, A. L.

Soukup, H., see Schropp, W.

Specht, A. W., see Hopkins, H. T.

Spencer, E. L., and Shive, J. W. (1933). *Bull. Torrey bot. Club* **60** : 423. The growth of *Rhododendron ponticum* in sand cultures. 5, 9, 21, 32, T19B, 103, 118, 143, 152

Spooner, E. C. R., see Howard, F.

Sprague, V. G., and Williams, E. M. (1941). *Plant Physiol.* **16** : 629. An inexpensive integrating light recorder. 163

Sprengel, C. (1839). "Die Lehre vom Dünger." Leipzig. Cited by Shive (1940a). 2

Spur, W. B., see Hayward, H. E.

Stahl, A. L., and Shive, J. W. (1933). *Soil Sci.* **35** : 375. Studies on nitrogen absorption from culture solutions 1. Oats. 13, 95, 119, 127, 139

Stahl, A. L., see Shive, J. W.

Steenbjerg, F., see Dorph-Petersen, K.

Steinberg, R. A. (1919). *Amer. J. Bot.* **6** : 330, 357. A study of some factors in the chemical stimulation of the growth of *Aspergillus niger*. 3, 4, 23, 67, 73

STEINBERG, R. A. (1930). *J. agric. Res.* **40** : 233. An automatic watering system with recorder for use in growing plants. 122

STEINBERG, R. A. (1931). *J. agric. Res.* **43** : 1071. An apparatus for growing plants under controlled environmental conditions. 161

STEINBERG, R. A. (1935). *J. agric. Res.* **51** : 413. Nutrient solution purification for removal of heavy metals in deficiency investigations with *Aspergillus niger*. 23, 73, 74

STEINBERG, R. A. (1936). *J. agric. Res.* **52** : 439. Relation of accessory growth substances to heavy metals including molybdenum in the nutrition of *Aspergillus niger*. 23, 52, 54, 66, 67, 68, 72, 76, 77, 94

STEINBERG, R. A. (1937). *J. agric. Res.* **55** : 891. Rôle of molybdenum in the utilisation of ammonium and nitrate nitrogen by *Aspergillus niger*. 65, 66, 67, 68, 76, 77, 94

STEINBERG, R. A. (1938a). *J. agric. Res.* **57** : 461. Applicability of nutrient solution purification to the study of trace element requirements of *Rhizobium* and *Azotobacter*. 68, 72, 76

STEINBERG, R. A. (1938b). *J. agric. Res.* **57** : 569. The essentiality of gallium to growth and reproduction of *Aspergillus niger*. 4, 52, 54, 67, 68, 75, 77, 110

STEINBERG, R. A. (1939). *J. agric. Res.* **59** : 731. Effects of nitrogen compounds and trace elements on growth of *Aspergillus niger*. 65, 67, 68, 94

STEINBERG, R. A. (1941). *J. agric. Res.* **62** : 423. Use of *Lemna* for nutrition studies on green plants. 4, 110, 154, 157

STEINBERG, R. A. (1943). *Chron. Bot.* **7** : 420. Use of *Lemna* as a test organism. 110, 154, 157

STEINBERG, R. A. (1945a). *Soil Sci.* **60** : 185. Use of micro-organisms to determine essentiality of minor elements. 4, 51, 54, 67, 68, 72, 76, 110, 154, 157

STEINBERG, R. A. (1945b). *Plant Physiol.* **21** : 42. Mineral requirements of *Lemna minor*. 54, 68, 76, 77, 154

STEINBERG, R. A. (1947a). *J. agric. Res.* **75** : 81. Growth responses to organic compounds by tobacco seedlings in aseptic culture. 115, 149

STEINBERG, R. A. (1947b). *J. agric. Res.* **75** : 199. Growth responses of tobacco seedlings in aseptic culture to diffusates of some common soil bacteria. 115, 149

STEINBERG, R. A. (1947c). *J. agric. Res.* **75** : 251. Effect of initial acidity on calcium and magnesium requirements of tobacco in aseptic culture. 149

STEPHENSON, R. B. (1942). *Plant Physiol.* **17** : 324. Sterilisation technique for grass seeds. 149

STEPHENSON, R. B. (1943). *Plant Physiol.* **18** : 37. Effects of certain growth regulating substances on growth correlation in lettuce seedlings. 149

STEUART, D. W., see CROWTHER, C.

STILES, W. (1915). *Ann. Bot. Lond.* **29** : 89. On the relation between the concentration of the nutrient solution and the growth of plants in water culture. 10, 88

STILES, W. (1916). *Ann. Bot. Lond.* **30** : 427. On the interpretation of the results of water culture experiments. 88

STILES, W. (1946). " Trace elements in plants and animals. University Press, Cambridge. 2

STILES, W., and JÖRGENSEN, I. (1917). *New Phytol.* **16** : 181. Observations on the influence of aeration of the nutrient solution in water culture experiments with some remarks on the water culture method. 133

STOKLASA, J. (1922). " Über die Verbreitung des Aluminiums in der Natur und seine Bedeutung beim Bau- und Betriebsstoffwechsel der Pflanzen." G. Fischer, Jena. (Cited by HUTCHINSON, G. E. (1945). *Soil Sci.* **60** : 29). 4

STOKLASA, J., SEBOR, J., and SEUFT, E. (1913). *Beih. bot. Zbl.* **30** : 167. Beitrag zur Kenntnis der Zusammensetzung des Chlorophylls. 104, 105

STONE, G. E. (1913). *Bull. Mass. agric. Exp.Stat.* **144**. The relation of light to greenhouse culture. 159

STOUGHTON, R. H. (1928). *J. sci. Instr.* **5** : 365. A method of maintaining constant humidity in closed chambers. 161

STOUGHTON, R. H. (1930). *Ann. appl. Biol.* **17** : 91. Apparatus for the growing of plants in a controlled environment. 161

STOUGHTON, R. H. (1941). *J. roy. hort. Soc.* **66** : 17. Soilless cultivation of plants. 143

STOUGHTON, R. H. (1947). *Agriculture* **53** : 539. Nutrient culture of crop plants. 143

STOUT, P. R., and ARNON, D. I. (1939). *Amer. J. Bot.* **26** : 144. Experimental methods for the study of the rôle of copper, manganese and zinc in the nutrition of higher plants. 11, 15, 17, 21, 22, 51, 52, 57, 62, 64, 65, 67, 68, 74, 75, 158

STOUT, P. R., and MEAGHER, W. R. (1948). *Science* **108** : 471. Studies of the molybdenum nutrition of plants with radioactive molybdenum. 79, 116, 168, 193

STOUT, P. R., OVERSTREET, R., JACOBSEN, L., and ULRICH, A. (1947). *Proc. Soil Sci. Soc. Amer.* **12** : 91. The use of radioactive tracers in plant nutrition studies. 15, 116, 117

STOUT, P. R., see ARNON, D. I.

STREET, H. E. (1950). *Ann. appl. Biol.* **37** : 149. Studies in plant nutrition II. Further studies of the effect of some organic supplements on the growth of plants in sand culture. 115

STREET, H. E., KENYON, A. E., and WATSON, G. M. (1946). *Ann. appl. Biol.* **33** : 369. The assimilation of ammonium and nitrate nitrogen by detached potato sprouts. 135, 149, 153

STREET, H. E., see BOLL, W. G.; CHESTERS, C. G. C.

STRICKLER, H. S., see WILSON, D. A.

STRONG, T. H., see TRUMBLE, H. C.

STRUCKMEYER, B. E., see MACVICAR, R.

STURGES, A. J., see FRAPS, G. S.

SWANBACK, T. S., see JACOBSEN, H. G. M.

TANADA, T. (1946). *J. agric. Res.* **72** : 245. Utilisation of nitrates by the coffee plant under different sunlight intensities. 11, T19B, 135, 143, 163

TARR, L. W., and NOBLE, E. L. (1922). *Bull. Del. agric. Exp. Stat.* **131**. The effect of hydrogen ion concentration upon the growth of seedlings. 10, 16, T19A, 99, 102, 149, 166

TAUBÖCK, K. (1942). *Bot. Ark.* (5th Suppl.) **43** : 291. From *Biol. Abs.* (D) 23 : 12531. The aluminium requirement of Pteridophytes. 19, 55

TAUSSON, W. O., PROKOVIEV, A. A., and PONTOVICH, W. E. (1944). *Doklady Akad. Nauk S.S.S.R.* **42** : 131. Sterile cultures as a method for studying metabolism in higher plants. 149, 150

TAVERNETTI, J. R., see GERICKE, W. F.

TEAKLE, L. J. H. (1929). *Plant Physiol.* **4** : 213. The absorption of phosphate from soil and solution cultures. 13, 87, 117, 124

TEMPLEMAN, W. G., see RICHARDS, F. J.

THARP, W. H., see WADLEIGH, C. H.

THELIN, G., and BEAUMONT, A. B. (1934). *J. Amer. Soc. Agron.* **26** : 1012. The effect of some forms of nitrogen on the growth and nitrogen content of wheat and rice plants. 95, 96, 108

THIMAN, K. V., and EDMONDSON, Y. H. (1949). *Arch. Biochem.* **22** : 33. The biogenesis of the anthocyanins. 1. General nutritional conditions leading to anthocyanin formation. 67, 149

THOMAS, L. S., HENDRIKS, R. H., and HILL, G. R. (1943a). *Plant Physiol.* **18** : 334. An installation of large sand culture beds surmounted by individual air conditioned greenhouses. 9, 23, T4, 26, 28, 48, 145, 162

THOMAS, L. S., HENDRIKS, R. H., and HILL, G. R. (1943b). *Plant Physiol.* **18** : 345. The utilisation of sulphate and sulphur dioxide for the sulphur nutrition of alfalfa. 48

THRESH, J. C. (1896). " Water and water supplies." London. 46

TIDMORE, J. W. (1930). *Soil Sci.* **30** : 13. Phosphate studies in solution cultures. 13, 87, 117, 144

TIEDJENS, V. A. (1934). *Plant Physiol.* **9** : 31. Factors affecting assimilation of ammonium and nitrate nitrogen, particularly in tomato and apple. 94, 100, 119

TIEDJENS, V. A., and BLAKE, M. A.(1932). *Bull. New Jersey agric. Exp. Stat.* **547**. Factors affecting the use of nitrate and ammonium nitrogen by apple trees. 93, 95, T19B, 103, 118, 141

TIEDJENS, V. A., and ROBBINS, W. R. (1931). *Bull. New Jersey agric. Exp. Stat.* **526**. The use of ammonia and nitrate nitrogen by certain crop plants. 93, 94, 95, 96

TINCKER, M. A. H. (1925). *Ann. appl. Biol.* **12** : 440. Physiological predetermination experiments with certain economic crops. The relation between rate of germination and subsequent growth. 165

TINCKER, M. A. H., see HICKS, F.

TINT, H. (1945). *Phytopathology* **35** : 511. An apparatus for the growth of plants under controlled temperature levels. 161

TOLLENS, B. J. (1882). *J. Landw.* **30** : 537. Ueber einige Erleichterungen bei der Kultur von Pflanzen in wässerigen Lösungen. T19A, 106, 107

TOTTINGHAM, W. E. (1914). *Physiol. Res.* **1** : 133. A quantitative chemical and physiological study of nutrient solutions for plant cultures. 2, 3, 10, 14, 16, 22, 83, 89, T19A, 151, 167

TOTTINGHAM, W. E. (1926). *Plant Physiol.* **1** : 307. Temperature effects in the metabolism of wheat. 161, 166

TOTTINGHAM, W. E., and RANKIN, E. J. (1923). *Amer. J. Bot.* **10** : 203. The availability of iron in nutrient solutions for wheat. 106

TOTTINGHAM, W. E., see LIVINGSTONE, B. E.

TREADWELL, D. W., see NIERECKER, R.

TRELEASE, S. F., and LIVINGSTONE, B. E. (1922). *Science* **55** : 483. Continuous renewal of solution for plants in water cultures. 120, 124, 126

TRELEASE, S. F., and TRELEASE, H. M. (1926). *Bull. Torrey bot. Club* **53** : 605. Relation of temperature to the physiological value of salt solutions as indicated by growth of wheat roots. 151

TRELEASE, S. F., and TRELEASE, H. M. (1933). *Science* **78** : 438. Physiologically balanced culture solutions with stable hydrogen ion concentration. 85, T19A, 100, 101

TRELEASE, S. F., and TRELEASE, H. M. (1935). *Amer. J. Bot.* **22** : 520. Changes in hydrogen ion concentration of culture solutions containing nitrate and ammonia nitrogen. T19B, 101, 103, 105

TRELEASE, S. F., and TRELEASE, H. M. (1938). *Amer. J. Bot.* **25** : 372. Selenium as a stimulating and possibly essential element for indicator plants. 4

TRENEL, M., and ALTEN, F. (1934). *Angew. Chem.* **47** : 813. Die physiologische Bedeutung der mineralischen Bodenazidität (worauf beruht die toxische Wirkung des Aluminiums ?). 25

TRUMBLE, H. C., and FERRES, H. M. (1946). *J. Aust. Inst. agric. Sci.* **12** : 32. Response of herbage legumes to applied nutrients on some southern Australian soils and their dependence on external factors. 18, 22, 51, 159

TRUMBLE, H. C., and SHAPTER, F. R. (1937). *Bull. Coun. sci. industr. Res. Aust.* **105**, pp. 25-36. Investigations on the associated growth of herbage plants. 2. The influence of nitrogen and phosphorus treatment on the yield and chemical composition of Wimmera rye grass and subterranean clover grown separately and in association. 28, 29, 32, T7, 40, 150

TRUMBLE, H. C., and STRONG, T. H. (1937). *Bull. Coun. sci. industr. Res. Aust.* **105**, pp. 11-24. Investigations on the associated growth of herbage plants. 1. On the nitrogen accretion of pasture grasses when grown in association with legumes. 28, 29, 32, T7, 150

TRUMBLE, H. C., see FERRES, H. M.

TURNER, W. I., and HENRY, V. M. (1939). "Growing plants in nutrient solutions." John Wiley, New York and Chapman & Hall, London. 87, 159

TWYMAN, E. S. (1945). Interim Report No. **7885**, to the Mineral Deficiencies Conference of the Agricultural Research Council. "The iron-manganese ratio in relation to the growth and development of oats with particular reference to the incidence of manganese deficiency." 66

TWYMAN, E. S. (1946). *New Phytol.* **45** : 18. The iron-manganese balance and its effect on the growth and development of plants. 108, 114

TYNER, E. H. (1935). *Soil Sci.* **39** : 405. The feeding power of plants for the potassium in feldspar, exchangeable form and dilute solution. 7, 9, 19, 25, 39, 41, 87, 120, 124

UHVITS, R., see HAYWARD, H. G.

ULRICH, A., see STOUT, P. R.

UNDERWOOD, L. M., see HALL, A. D.

URQUART, D., see MYERS, R. J.

VANDECAVEYE, S. C., see RAMIG, R. E.

VANSELOW, A. P., and DATTA, N. P. (1949). *Soil Sci.* **67** : 363. Molybdenum deficiency of the citrus plant. 15, 17, 22, 24, 55, 65, 66, 68, 76, 82, 114, 137, 151, 152

VANSELOW, A. P., see CHAPMAN, D. H. ; LIEBIG, G. F. Jr.

VANTSIS, T. U., and BOND, G. (1950). *Ann. appl. Biol.* **37** : 159. The effect of charcoal on the growth of leguminous plants in sand culture. 40

VEAL, M. P., see HENDERSON, J. H. M.

VAN DER VEEN, R. (1940). *Arch. Koffiecult.* **14** : 199. Voedingsphysiologische onderzoekingen bij koffee III. 95, T19B, 99, 107, 143, 151

VICKERY, H. B., LEAVENWORTH, C. S., and BLISS, C. I. (1949). *Plant Physiol.* **24** : 335. The problem of selecting uniform samples of leaves. 169

VICKERY, H. B., see PUCHER, G. W.

VICKERY, R. C. (1946). *Nature* **158** : 623. Adsorption on carbon of rare earth organic complexes. 72

VIRTANEN, A. I. (1933). *Herb. Rev.* **1** : 88. The nitrogen nutrition of plants. 150, 165

VIRTANEN, A. I. (1949). *Experientia* **8** : 313. On the rôle of substances present in the seeds and arising in them during germination in the growth of plants. 115, 164

VIRTANEN, A. I., and v. HAUSEN, S. S. (1931a). *Biochem. Z.* **232** : 1. Untersuchungen über die Leguminosen-Bakterien und-Pflanzen. IX. 150

VIRTANEN, A. I., and v. HAUSEN, S. S. (1931b). *Z. PflErnähr. Düng.* A **21** : 57. Untersuchungen über die Leguminosen-Bakterien und-Pflanzen X. Über die Tätigkeit der Leguminosenbakterien und die Ausnutzung des in Wurzelknöllchen der Leguminosen gebundenen Stickstoffs durch Nichtleguminosen. 150

VIRTANEN, A. I., and v. HAUSEN, S. S. (1949a). *Nature* **163** : 482. Rôle of substances found during germination in the growth of plants. 115, 164

VIRTANEN, A. I., and v. HAUSEN, S. S. (1949b). *Z. PflErnähr. Düng.* **90** (n.s. **45**) : 11. Über die Bedeutung der das Redoxpotential erniedrigenden Stoffe für das Wachstum der Pflanzen. 115, 164

VIRTANEN, A. I., v. HAUSEN, S. S., and KARSTROM, H. (1933). *Biochem. Z.* **258** : 106. Untersuchungen über die Leguminosen-Bakterien und-Pflanzen. XII. Die Ausnutzung der aus den Wurzelknöllchen der Leguminosen herausdiffundierten Stickstoffverbindungen durch Nichtleguminosen. 149, 150

VIRTANEN, A. I., and LINKOLA, H. (1946). *Nature* **158** : 515. Organic nitrogen compounds as nitrogen nutrition for higher plants. 98

VLAMIS, J., and DAVIS, A. R. (1943). *Plant Physiol.* **18** : 685. Germination, growth and respiration of rice and barley seedlings at low oxygen tensions. 133

VLAMIS, J., and DAVIS, A. R. (1944). *Plant Physiol.* **19** : 33. Effects of oxygen tension on certain physiological responses of rice, barley and tomato. 133

VOGEL, F. (1929). *Gartenbauwiss.* **2** : 351. Zur Technik der Anstellung von Gefässvegetationsversuchen im Gartenbau. 18, 47, 48, 147

VOGEL, F. (1931). *Gartenbauwiss.* **5** : 35. Zur Technik der Anstellung von Gefässvegetationsversuchen im Gartenbau. 18, 47, 48, 147

VOLK, N. J., see HOLT, M. E.

VOTH, P. O., and HAMNER, K. C. (1940). *Bot. Gaz.* **102** : 169. Response of *Marchantia polymorpha* to nutrient supply and photoperiod. 154

WADE, G. C. (1944). *J. Aust. Inst. agric. Sci.* **10** : 176. Production of copper deficiency symptoms in citrus in solution cultures. 11, 17, 22, 23, 54, 75, 135, 143, 152

WADLEIGH, C. H. (1939). *Soil Sci.* **48** : 109. The influence of varying cation proportions upon the growth of young cotton plants. 167

WADLEIGH, C. H., ROBBINS, W. R., and BECKENBACH, J. R. (1937). *Soil Sci.* **43** : 153. The relation between the chemical nature of the substrate and the degree of chlorosis in corn. 9, 21, 90, 100

WADLEIGH, C. H., and THARP, W. H. (1940). *Bull. Ark. agric. Exp. Stat.* **401**. Factorial design in plant nutrition experiments in the greenhouse. 166

WADLEIGH, C. H., see BECKENBACH, J. R. ; BOWER, C. A. ; GAUCH, H. G. ; MAGISTAD, O. C.

WAGNER, F. (1940). *Phytopath. Z.* **12** : 427. Die Bedeutung der Kieselsaüre für das Wachstum einiger Kulturpflanzen, ihren Nährstoffhaushalt und ihre Anfälligkeit gegen echte Meltaupilze. 4, 22, 25, 51, T19B

WALKER, J. C. (1941). *J. agric. Res.* **62** : 573. The boron deficiency disease in cabbage. 155

WALKER, J. C., see GALLEGLEY, M. E.

WALKLEY, A. (1940). *J. Coun. sci. industr. Res. Aust.* **13** : 255. The zinc content of some Australian fertilisers. 64, 65, 66

WALKLEY, A. (1942). *Aust. J. exp. Med.* **20** : 139. The determination of zinc in plant materials. 23, 51, 52, 54, 65

WALL, M. E. (1940). *Soil Sci.* **49** : 315. The rôle of K in plants. II. Effect of varying amounts of K on the growth status and metabolism of tomato plants. 84, 87

WALL, R. F., and HARTMAN, E. L. (1942). *Proc. Amer. Soc. hort. Sci.* **40** : 460. Sand culture studies on the effects of added salts upon the composition of tomato plants. 91

WALLACE, R. H., and BUSHELL, R. J. (1945). *Plant Physiol.* **20** : 443. A simple and effective humidity control. 161

WALLACE, T. (unpublished work). 159

WALLACE, T. (1922). *A.R. Long Ashton agric. hort. Res. Stat.* 1921, p. 42. Pot experiments on the manuring of fruit crops. 1st Report. 5, 6, 7, 19, 48, 141, 153

WALLACE, T. (1924). *J. Pomol.* **4** : 117. Experiments on the manuring of fruit trees. 5, 6, 15, 16, 19, T4, 28, 44, 48, 85, T19B, 118, 141, 153, 165

WALLACE, T. (1925). *J. Pomol.* **5** : 1. Experiments on the manuring of fruit trees II. 16

WALLACE, T. (1928). *J. Pomol.* **6** : 243. Leaf scorch on fruit trees. 15, 19, 48, 118, 165

WALLACE, T. (1930). *J. Pomol.* **8** : 23. Experiments on the manuring of fruit trees III. The effects of deficiencies of potassium, calcium and magnesium, respectively, on the contents of these elements, and of phosphorus in the shoot and trunk regions of apple trees. 5, 6, 8, 15, 16, 29, 48, 85, T19B, 141, 150, 153, 159

WALLACE, T. (1932). *Forestry* **6** : 9. The nutrition of woody plants (with special reference to cultivated fruit plants) (also reproduced in *Tech. Commun. Bur. Fruit Prod.* 4). 143

WALLACE, T. (1933). *Occ. Paper Imp. Bur. Fruit Prod.* **1**. Technique in pot culture for fruit plants. 171

WALLACE, T. (1943). The diagnosis of mineral deficiencies in plants. A colour atlas and guide. H.M.S.O. London, 1943 (with supplement 1944). 141

WALLACE, T., and HEWITT, E. J. (1946). *J. Pomol.* **22** : 153. Studies in iron deficiency of crops. 1. Problems of iron deficiency and the interrelationship of mineral elements in iron nutrition. 114

WALLACE, T., and HEWITT, E. J. (1947). *Nature* **161** : 28. Effects of calcium deficiency on potato sets in acid soils. 164

WALTMAN, C. S. (1931). *Bull. Ky agric. Exp. Stat.* **321**. Effect of hydrogen ion concentration on the growth of strawberries in sand and in soil. 28, T7, T19B, 98, 99, 153

WALTMAN, C. S. (1940). *Bull. Ky agric. Exp. Stat.* **410**. The effect of nitrogen and phosphorus on the growth of apple and peach trees in sand culture 8, 16, T19B, 141

WANDER, I. W., see BITCOVER, E. H.

WANN, F. B., see HOPKINS, E. F.

WARING, W. S., and WERKMAN, C. H. (1942). *Arch. Biochem.* **1** : 303. Growth of bacteria in an iron-free medium. 22, 23, 54, 71, 75, 81

WARINGTON, K. (1923). *Ann. Bot. Lond.* **27** : 629. The effect of boric acid and borax on the broad bean and certain other plants. 3, 10, 67, 83, 112

WARINGTON, K. (1933). *Ann. Bot. Lond.* **47** : 429. The influence of length of day on the response of plants to boron. 159

WARINGTON, K. (1934). *Ann. Bot. Lond.* **48** : 743. Studies in the absorption of calcium from nutrient solutions with special reference to the presence or absence of boron. 86

WARINGTON, K. (1937). *Ann. appl. Biol.* **24** : 475. Observations on the effect of molybdenum on plants with special reference to the *Solanaceae*. 83, 114

WARINGTON, K. (1946). *Ann. appl. Biol.* **33** : 249. Molybdenum as a factor in the nutrition of lettuce. 10, 52, 65, 67, 111, 114, 151

WARINGTON, K., see BRENCHLEY, W. E.

WARINGTON, R., see LAWES, J. B.

WATSON, D. J. (1947). *Ann. Bot. Lond.* **11** n.s. : **41**. Comparative physiological studies on the growth of field crops. 168

WATSON, G. M., see STREET, H. E.

WATSON, S. A., and NOGGLE, G. R. (1947). *Plant Physiol.* **22** : 228. Effect of mineral deficiencies on the synthesis of riboflavin and ascorbic acid by the oat plant. 131

WAUGH, J. G., see CULLINAN, F. P.

WEATHERWAX, P. (1914). *Proc. Indiana Acad. Sci.* 1914, p. 157. An apparatus for aerating culture solutions. 135

WEINBERGER, J. H., and CULLINAN, F. P. (1936). *Proc. Amer. Soc. hort. Sci.* **34** : 249. Symptoms of some mineral deficiencies in one-year Elberta peach trees. 31, T7, T19B, 118, 141

WEISS, M. G. (1943). *Genetics* **28** : 253. Inheritance and physiology of efficiency in iron utilisation in soya beans. 165

WEISSMANN, H., see LEMMERMAN, O.

WELCHER, F. J. (1947). "Organic analytical reagents." 4 volumes, Van Nostrand, New York. 80, 195

WELLS, J. E., and PEMBERTON, R. (1947). *Analyst* **72** : 185. The microchemical determination of molybdenum in steel. 81

WENGER, P., and DUCKERT, R. (1941). *Helvetica chim. Acta* **24** : 1143. A critical study of the qualitative reagents for cations IV. Manganese. 62, 69

WENT, F. W. (1943a). *Plant Physiol.* **18** : 51. Effect of the root system on tomato stem growth. 24, 155

WENT, F. W. (1943b). *Amer. J. Bot.* **30** : 157. Plant growth under controlled conditions. 1. The air-conditioned greenhouses at the California Institute of Technology. 161, 162, 166

WENT, F. W. (1944). *Amer. J. Bot.* **31** : 135. Plant growth under controlled conditions II. Thermoperiodicity in growth and fruiting of tomato. 160

WERKMAN, C. H., see WARING, W. E.

WEST, C., BRIGGS, G. E., and KIDD, F. (1920). *New Phytol.* **19** : 200. Methods and significant relations in the quantitative analysis of growth. 168

WEST, C., see BRIGGS, G. E. ; KIDD, F.

WHEELER, H. J., see HARTWELL, B. L.

WHITCHER, E. J., see HEATH, O. V. S.

WHITE, D. G., and CHILDERS, N. F. (1944). *Plant Physiol.* **19** : 699. A method for measuring root respiration. 137

WHITTENBERGER, R. T. (1945). *Amer. J. Bot.* **32** : 539. Silicon absorption by rye and sunflower. 26

WIEGMAN and POLSTORF. Given by REED (1942). 2

WILLIAMS, E. M., see SPRAGUE, V. G.

WILLIAMS, R. F. (1946). *Ann. Bot. Lond.* **10** n.s. : 41. The physiology of plant growth with special reference to the net assimilation rate. 168

WILSON, A. R. (1937). *Ann. appl. Biol.* **24** : 911. Apparatus for growing plants under controlled environmental conditions. 161

WILSON, D. A., and STRICKLER, H. S. (1939). *Industr. Engng Chem.* (*Anal.*) **11** : 636. Constant level still for redistillation of water. 55

WILSON, J. K. (1915). *Amer. J. Bot.* **2** : 420. Calcium hypochlorite as a seed steriliser. 149, 150

WILSON, P. W., see HOPKINS, E. W.

WILSON, R. D. (1949). *Aust. J. Sci.* **11** : 209. Molybdenum in relation to the scald disease of beans. 164

WINTER, E. J. (private communications). 6, 27

WITHROW, A. P., and WITHROW, R. B. (1947). *Plant Physiol.* **22** : 494. Plant growth with artificial sources of radiant energy. 162

WITHROW, R. B., and BIEBEL, J. P. (1936). *J. agric. Res.* **53** : 693. A sub-irrigation method of supplying nutrient solutions to plants growing under commercial and experimental conditions. 18, 19, 26, 143

WITHROW, R. B., and WITHROW, A. P. (1948). *Stat. Circ. Purdue Univ. agric. Exp. Stat.* **328**. Nutriculture. 18, 50, 144

WITTWER, S. H., SCHROEDER, R. A., and ALBRECHT, W. A. (1947). *Plant Physiol.* **22** : 244. Interrelationship of calcium, nitrogen and phosphorus in vegetable crops. 35, 38

WOLF, E. T. (1866). *Landw. VersSta.* **8** : 189. Ueppige Vegetation in wasserigen Lösungen der Nährstoffe. 3, 11

WOLFE, E., and KNOP, W. W. Given by PEARSE (1940). 141

WOLFE, H. S. (1926). *Bot. Gaz.* **81** : 228. The auximone question. 115

WOLFF, L. K., and EMMERIE, A. (1930). *Biochem. Z.* **228** : 443. Über das Wachstum des *Aspergillus niger* und den Kupfergehalt des Nährbodens. 82

WOLKOFF, M. L. (1918). *Soil Sci.* **5** : 123. Effect of ammonium sulphate in nutrient solution on the growth of soya beans in sand cultures. 5, 6, 7, 95, 102, 137

WOOD, J. G., and HONE, M. R. (1948). *Aust. J. sci. Res. ser.* B. **1** : 163. Studies on the nitrogen metabolism of plants VIII. Utilisation of α-oximino-carboxylic acids by oat plants. 98

WOODCOCK, H. H. D. (1946). *J. roy. hort. Soc.* **71** : 326. Recent developments in lily production. 26

WOODFORD, E. K., and GREGORY, F. G. (1948). *Ann. Bot. Lond.* **12** n.s. : 335. Preliminary results obtained with an apparatus for the study of salt uptake and root respiration of whole plants. 138, 140, 168

WOODFORD, E. K., see GREGORY, F. G.

WOODMAN, R. M. (1939a). *Ann. Bot. Lond.* **3** n.s. : 649. Studies in the nutrition of vegetables. The effects of variation in the nitrogen supply on lettuce (v. May King) in sand culture. 8, 21

WOODMAN, R. M. (1939b). *J. agric. Sci.* **29** : 229. Studies in the nutrition of vegetables. Phosphate deficiency and yield tests on sand cultures of May King lettuce. 5, 8, T4, 27, 88, 150, 166

WOODMAN, R. M. (1939c). *J. Pomol.* **17** : 167. Effects of variation in the supply of potash to lettuces grown under glass. 19

WOODMAN, R. M. (1940a). *Ann. appl. Biol.* **27** : 5. The nutrition of lettuces grown in sand cultures under glass. 7, 21, 88

WOODMAN, R. M. (1940b). *Ann. appl. Biol.* **27** : 445. The effect of the concentration of the culture solution on vegetables grown in sand. 7, 19, 88

WOODMAN, R. M. (1941a). *Ann. appl. Biol.* **28** : 1. The nutrition of turnips. 19, 21, 88

WOODMAN, R. M., (1941b). *Ann. appl. Biol.* **28** : 181. The nutrition of spring cabbages. 8, 21, 88

WOODMAN, R. M. (1942). *Ann. appl. Biol.* **29** : 97. The nutrition of spinach. 88

WOODMAN, R. M. (1943a). *Ann. appl. Biol.* **30** : 1. The nutrition of the carrot. 88

WOODMAN, R. M. (1943b). *Ann. appl. Biol.* **30** : 116. Nitrogen nutrition of the onion. 88

WOODMAN, R. M. (1943c). *Ann. appl. Biol.* **30** : 319. The nutrition of the radish. 88

WOODMAN, R. M. (1944a). *Ann. appl. Biol.* **31** : 19. The nutrition of the pea. 21

WOODMAN, R. M. (1944b). *Ann. appl. Biol.* **31** : 22. The nutrition of vegetables in sand. 21, 88

Woodman, R. M. (1946). *J. agric. Sci.* **36** : 69. Plant growth with nutrient solutions. 1. A brief review of existing work. 2, 167

Woodward, J. (1699). *Phil. Trans. roy. Soc. (London)* **21** : 193. Thoughts and experiments on vegetation. 2, 42

Wright, E. L., see Ferguson, W.

Wright, K. E. (1937). *Plant Physiol.* **12** : 173. Effects of phosphorus and lime in reducing aluminium toxicity of acid soils. 25

Wynd, F. L. (1933). *Ann. Mo. bot. Gdn* **20** : 223. Sensitivity of orchid seedlings to nutritional ions. T19B

Yates, F. 166

Yates, F. (1937). *Tech. Commun. Bur. Soil Sci. Rothamsted* **35**. " Design and analysis of factorial experiments." 167

Yoshimura, F. (1934). *J. Fac. Sci. Hokkaido Imp. Univ. Sapporo,* Ser. Vth 3, **89**. Spherical cell formation in *Aspergillus oryzae* with special reference to heavy metal impurities in culture solution. 66, 73

Youden, W. J. (1940). *Contr. Boyce Thompson Inst.* **11** : 219. Experimental design to increase accuracy of greenhouse studies. 166

Young, E. G., Begg, R. W., and Pentz, E. I. (1944). *Arch. Biochem.* **5** : 121. The inorganic nutrient requirements of *Escheria coli*. 68

Young, H. Y., see Sideris, C. P.

Young, J. B., see MacIntyre, W. H.

Young, R. S. (1935). *Bull. Cornell agric. Exp. Stat.* **174**. Certain rare elements in soils and fertilisers and their rôle in plant growth. 64, 65

Zentmeyer, G. A. (1944). *Science* **100** n.s. : 294. Inhibition of metal catalysis as a fungistatic mechanism. 80

Zimmerman, P. W., and Berg, R. O. (1934). *Contr. Boyce Thompson Inst.* **6** : 39. Effects of chlorinated water on land plants, aquatic plants and goldfish. 50

Zinzadze, Ch. (1926). *Landw. VersSta.* **105** : 267. Neue normale Nährlösungen mit stabilen Reaktionen. 11, 31, 32, T19A, 100, 101, 104, 105

Zinzadze, Ch. (1932-1933). *Ann. agron. Paris* **2** : 809 and **3** : 53. Nutrition artificielle des plantes cultivées. I. Mélanges nutritifs à pH stabile. 13, 15

Zinzadze, Ch. (1935). *Science* **81** n.s. : 540. A new arrangement for regulating the flow of liquid into a culture vessel. 122, 124, 125

Zoller, E., see Schropp, W.

Zurbicki, Z. (1933). *Plant Physiol.* **8** : 553. New method of arranging experiments with vegetables in sand cultures with flowing solution. 124

SUBJECT INDEX

(Letter T signifies Table number)

A

Absorption of nutrients
— effects of aeration, 131, 132, 139, 140
— — age, 86, 139
— — nitrogen supply, 95, 96
— — concentration, 139, 140
— — genetic characters, 165
— — pH, 93, 94, 98, 99, 139
— — rootstock, 165
— — salt status, 92, 139, 140
— — sand culture, 88
— — temperature, 91, 140, 141
— from ion exchange materials, see Ion exchange cultures
— from solid compounds, see Nutrient supply
— measurement of, 8, 10, 11, 12, 13, 137, 138-141
— see also Iron, respective nitrogen compounds
Acetamide, 97
Activated carbon, see Nutrient reagents, Water purification
Adsorption of nutrients on charcoal, 40
see also Nutrient reagents, Sand
Aeration
— effects of lack of, T19, 99, 131, 133
— — excess of, 133
— methods for supplying, 134, 135, 137, 139, 140, 150
— need for, 3, 14, 127, 129, 131, 133
— oxygen tension, 4, 131, 133, 137, 138
— requirements in relation to
carbon dioxide level, 132
crop, 14, T19, 131, 133, 135
culture vessel, 10-13, 14, 135
drainage, 4, 118, 119
frequency, 135
nitrification, 97, 133
micronutrients, 94, T19
nitrogen supply, 94, T19
sand culture, 4, 27, 119, 127, 129, 133, 154
solution renewal, T19, 119, 127-129, 135
temperature, 135
— root atmosphere studies, 137, 140
— root rots, 99, 131, 135
— sealed cultures, 137, 138, 140, 154
Air-conditioned greenhouses, 162
Alanine, 97, 98
Alfalfa, see Lucerne
Algal cultures, growth, control of, 28, 120, 131, 161
Alloxan, 97
Almond, 2, 141
Alternating solution treatments, see Renewal of Solution
Aluminium
— as a plant nutrient, 1, 4, 19, 55, T19, 108, 109, 110, 115, 152, 188, 189
— freedom from gallium, 109
— in sand or water, 29, 44
— paint, use of, 15, 159
— removal of, 76
— toxicity, 25, 48, 156, 190
Amides, 97, 98
Amidosulphonic acid, 97
Amines, 97
Amino acids, 97, 98
Ammonium compounds
— absorption, effect of age, 95
— — effect of pH, 93, 94, 139
— — effect on pH, 94, 100, 101
— — effect on requirements for aeration, 94, 133, 139
— — calcium, 3, 95, 96, 98
— — iron, 96, 190
— — micronutrients, 94
— — comparison with nitrate, 93-96, 97, 98, 100, 139
— compounds used, 95, 96, 97, 101
— concentrations supplied, 86, T19, 101
— impurities in, 66
— injury from, 95, 96, 98, 100, 101, 190
— nitrification of, 98, 149
— purification of, 74
— ratio to nitrate, 101
— tolerance to, in relation to crop, 95, 96, 97, 98
— — — light, 94
— — — season, 94
— — — solution renewal, 94
Apple, 7, 141, 152, 153, 161
— aeration in water culture, 106, 131, 142, 165
— growth in sand culture, 5, 6, 7, 8, 9, 48, 87, 88, T19, 100, 118, 141, 149
— growth in water culture, 12, T19, 100, 106, 122, 124, 141, 142
— nutrient requirements, 87, 106, 141
Applicability of methods, 169-170
Apricot, 12, 71
Ascorbic acid, 115, 116
Aspartic acid, 97, 98
Asparagine, 97, 98
Aspergillus niger, 4, 54, 55, 67, 82
— as a test organism, 67, 68, 76, 77, 79, 157
— micronutrient deficiency techniques, 54, 67, 71, 72, 73, 75, 154
Asphalt, see Bitumen
Associated growth cultures, 24, 32, 40, 150
Astragalus, 4
Atomised culture solutions, see Renewal of solution
Automatic control of nutrient supply or water
—, see Renewal of solution
Auximones, see Organic growth supplements
Avocado, T19, 97, 112, 133, 153
A-Z treatments, see Micronutrients
Azotobacter, 4, 73, 76

B

Bacteria, 75, 81, see also *Azotobacter*, *Escheria*, *Rhizobium*
Balsam, 133
Barbituric acid, 97
Barium, 29
Barley, 5, 7, 10, 11, 12, T4, 25, 27, 35, 37, 39, 40, 41, 50, 51, 52, 87, 92, 93, 94, 96, T19, 97, 98, 105, 107, 112, 113, 127, 133, 139, 140, 149, 156, 161, 163, 165
Base exchange, see Ion exchange

Bean, 67, 91, 101, 102, 115, 131, 150, 151, 163, 164, 198, 200
— broad, 10, 57, 66, 96, 114
— French (dwarf, kidney, runner), 36, 57, 147, 156
— Soya, 5, 7, 10, 13, 36, 38, 39, 86, 87, 96, 98, 105, 107, 108, 111, 113, 124, 127, 129, 132, 135, 149, 156, 159, 163, 164, 165
Beet, 4, 6, 7, T4, 48, 50, 57, 66, 70, 91, 93, 112, 151, 152, 198, 199, see also Sugar beet
Beltsville, U.S. Dep. Agric., 162
Bentonite, see Ion exchange cultures
Bermuda grass, 12, 13, 98, 107, 112
Bidens cannabina, 2
Bird cages, 145
Bitumen and asphalt materials
— emulsions, 18, 144
— mineral content of, 18, 47, 172
— paint, application to clay pots, 20, 158, 172-173
— — — concrete, 18-19, 147
— — — galvanised surfaces, 18, 46, 47, 145
— — — iron surfaces, 17, 18, 47, 147
— permeability, 47
— stability, 47
— toxicity, 18, 19
Blackberry, 112
Blackcurrant, 118, 151
Bluebell, 159
Boron
— in containers, 18, 20, 21, 172
— in nutrient reagents, 64-66, 72
— in sand, 29, T7, 30, 33
— in water, 43, 45, 46, 47, 48, 49, 51, 52, 57, 59, 60, 61
— deficiency, 111-112, 143, 147, 159
— — techniques, 17, 20, 21, 52, 151, 156, 158, 173, 199, 200
— need of, 3, 111, 157, 165
— normal levels, T19, 111-112, 189, 190
— sources of, 114
— tests for, biological, 7, 165
— — chemical, 67, 68, 196-197
— toxicity, 48, 112, 143, 159
Boyce Thompson Institute, 162
Brassica crops, 6, 7, 9, 10, 13, 48, 57, 112, 115, 151, 152, 156, 157, 164, 165, 191, 198, 200
Bromine, 108, 109, 110
Bryophyllum, 101
Buckwheat, 5, 6, 7, 10, 13, T4, 25, 87, 89, 94, 96, 98, 105, 133, 149, 156

C

Cabbage, 8, 10, 50
Cacao, 4, 5, 10, 17, 27, 124, 129, 133, 143, 153, 156
Cadmium, 64, 79, 108, 109, 110
Calcium absorption
— effect of ammonium ion on, 95
— effect of pH on, 98
— from carbonate, 41, 190
— — containers, 19, 172
— — ion-exchange cultures, 35-39
— — or in sand, 30, 31, 32, T7, 33
— compounds, impurities in, 65, 66
— — purification of, 70-82
— deficiency, 37, 38, 157, 198
— normal levels, 84-86, 87, T19, 189, 190
California, University of, 53, 76
— Riverside, Citrus research station at, 144
Cantaloupe, 149
Carbon, activated, see Nutrient reagents and Water purification
Carbon dioxide, limitation of, 162, see also Aeration
Carnation, T4
Carrot, 91, 98, 200
Cauliflower, 6, 27, 41, 57, 92, 97, 98, 112, 157, 165, 199, 200
Celery, 92, 198, 200
Cereals, 3, 6, 7, 9, 13, 25, 57, 70, 92, 108, 112, 113, 114, 115, 133, 151, 152, 156, 157, 161, 164, 200
Change of treatment, 155, 199
Charcoal admixed in sand cultures, 40
— see also Nutrient reagents and Water Purification
Chard, 157
Chestnut, 2, 141
Chlorella, 74
Chlorine, 1, 4, 50, T19, 109, 110
Chlorosis, control of, T19, 102-108, 144, 155
— incidence in sand culture, 102-108, 155
— — water culture, 94, T19, 103-108, 113, 144, 155
— induced by heavy metal excess, 108, 111, 113, 114
— relation to crop, T19, 103, 104, 105, 106, 113, 144
— — culture vessel, 101, 104, 155
— — light, 159
— — nitrogen supply, 94, 95, 103, 111
— — pH, 95, 99, 102, 103
— — see also Iron
Chromium, 29, 76, 103, 108, 109, 110
Chrysanthemum, 159
Circulation of solution, see Renewal of solution
Citrus, 4, 39, 57, 67, 76, 107, 111, 119, 141, 151, 152
— aeration of, 97, 131, 133, 135, 143, 144, 165
— nutrient requirements, 87, T19, 102, 103, 111-114, 143, 144
— raising culture plants, 143
— sand cultures, 8, 10, T4, 88, T19, 97, 99, 102, 103, 112, 113, 129, 143
— water cultures, 11, 12, 13, 51, 87, 90, T19, 97, 99, 113, 122, 143, 144
— see also grapefruit, lemon, orange
Clover, 7, 25, 28, 57, 87, 95, 97, 98, 112, 115, 131, 149, 150, 151, 156, 164, 198, 199, 200
Cobalt, 1, 4, 65, 66, 71, 76, 80, 81, 82, 108, 109, 110, 188, 189
Coffee, 11, 95, T19, 99, 107, 108, 135, 143, 152, 163
Colloidal clay, see Ion-exchange cultures
Comparison of sand and water cultures, 6, 31, 90, 100, 104, 113, 117, 127-128, 135, 154-156
Coniferous trees, 143, see also Pine, *Pinus*
Constant level cultures, see Renewal of solution
Containers, capacity, 5-14, 143-147, 172, 173, 198
— cleaning, 23, 24, 173, 196
— composition of ; clay, glazed, 20-21, 174
— — — unglazed, 19, 172, 173
— — concrete, 18-19, 144, 145, 146, 147, 148
— — enamelled, 18
— — glass, 22-24, 145, 173
— — metal, 17-18, 144, 145, 147
— — plastic, 19, 173
— — wood, 19, 147
— contamination from, 18, 19, 20, 21, 22, 173
— covers for, 15-17
— drainage from, 4, 18, 19, 131, 144, 145, 146, 148, 172
— exclusion of light from, 15, 173
— large-scale cultures, 6, 8, 9, 12, 13, 144-148
— multiple compartment, 24, 25
— retention of nutrients by, 20, 21

Containers, shape, 14-15
— for tree cultures, 18, 144-147
Copper, contamination in containers, 17
— — in nutrient reagents, 64-66, 103
— — in sand, 29, T7, 31, 33
— — in water, 44, 45, 46, 47, 49, 51, 55, 57, 59, 60
— deficiency, T19, 105, 113, 157
— — techniques, 17, 74, 75, 151, 156, 158, 179, 191, 200
— need of, 4, 112-113
— normal levels, T19, 111, 112-113, 189, 190, 191
— removal, see Nutrient reagents
— tests for, 68, 78
— toxicity in water or solutions, 51, 52, T19, 108, 112, 113, 155
Cotton, 9, 13, 50, 87, 92, 97, 112, 163
Crop, choice of, 156-157, 200
— indicator, 199, 200
— pure line, 156, 165
— variety, 156
— see also Raising culture plants and respective crops
Cucumber, 157
Culture vessels, see Containers
Cyanuric acid, 97

D

Dallis grass, 36
Deficiencies
— demonstration of, 198, 200
— time interval for onset of, 199
— see also respective elements
Design of experiments, 89, 165-168
— codes for identification of treatments, 167, 197
— factorial designs, 166, 170
— layout, 156, 166, 168
— movement of cultures, 166
— randomised layouts, 166, 197
— study of independent ion effects and interactions, 90, 167, 168
— systematic layouts, 166, 197
— triangular and polyhedral designs, 3, 89, 90, 167
Dialysed clays, see Ion-exchange cultures
Diphenylthiocarbazone (dithizone) test, 54, 57, 62, 68, 74, 78, 80, 191, 193, 196
Diseases, see Pest control
Drip cultures, see Renewal of solution
Dust, contamination from, 15

E

Enamel, see Containers
Environment, controlled, 140, 141, 161-162
— effects on culture plants, 91, 140, 159-160, 169, 170
— see also Design, Humidity, Light, Revolving table, Temperature
Escheria coli, 68
Essential elements, criteria of, 4

F

Flax, 10, 11, 24, 25, 57, 70, 91, 96, T19, 104, 105, 108, 119, 131, 145, 152, 199
Fluorine, T19, 109, 110
Formamide, 97

G

Gallium, 1, 4, 17, 68, 71, 72, 75, 76, 77, 79, 80, 109, 110, 111, 115, 152, 188
Galvanised containers and tanks, see Containers, Toxicity, Water, Zinc
Glass
— cleaning, 23, 24, 173, 196, 199
— contamination by boron, 22, 52
— — manganese, 22
— — zinc, 22, 23, 52
— containers, see Containers
— hysil, 23, 52, 158, 181
— pyrex, 21, 22, 23, 41, 47, 51, 52, 53, 54, 55, 56, 114, 137, 158, 173, 181, 186, 187, 199
— resistance, soda, miscellaneous, 22, 23, 52
— wool, 5
Glaze, see Containers
Glutamic acid, 97, 98
Glutamine, 98
Glutathione, 115
Glycine, 97, 98
Gooseberry, 118
Grapefruit, 152
Grass, 97, 115, see also respective species
Growth, measurement of, 168, 169
— seasonal effect on, 161
— variability, causes of, 165
— — extent of, 156, 161, 165
Guanidine hydrochloride, 97, 98

H

Heavy metals
— in sand or water, 29, 52, 54, 55, 57, 62
— test for, 52, 54, 55, 57, 62, 68, 69, 74, 185-186, 196
— toxicity from, 108, 111, 114, 147, 200
— see also Humate, Nutrient reagents
Hemp, 105
Hexamethylene tetramine, 97
Hippuric acid, 97
Histidine, 97
Hop, 5, 8
Humate metals and micronutrients, T19, 99, 106, 107, 112, 114, 115
— see also iron
Humidity, control of, 161, 162
— effects of, 160
— normal limits of, 160, 162
Hydrazine, 97
Hydrogen ion concentration, see pH
Hydroxylamine, 97, 98
8-Hydroxyquinoline (oxine), 78, 79, 80, T18, 192, 193, 195, 196
Hysil, see Glass

I

Independent ion effect, see Design, Nutrient supply
Iodine (and iodide), 48, 80, T19, 109, 110, 111
Ion-exchange cultures
— absorption from clays, 32, 34, 35, 36, 38-39, 99, 124
— — ion exchange resins, 34, 36-38
— — kaolin, 39
— — zeolites, 32, 35
— calcium uptake from and saturation of, 36, 37, 38, 39
— comparative tests with sand or water cultures, 36, 37, 38

Ion-exchange cultures *contd.*
— electrodialysis, 35
— exchange capacities, 34, 35, 39
— large-scale application, 8, 36, 37
— micronutrient supply, 36, 37, 99, 108, 114
— Preparation of, 34-40
Ion-exchange resins, see Ion-exchange cultures, Nutrient reagents, Water
Iron
— compounds, impurities in, 66, 102, 105
— concentrations used, T19, 103-108, 188, 189
— contamination by, in containers, 21, 174
— — nutrient reagents, 64, 68, 81
— — sand, 29, 30, T7, 33
— — water, 44, 45, 46, 47, 49, 51, 49, 60, 61
— deficiency of, induced by carbonates, 41, 103
— — heavy metals, 108, 114
— — phosphate, 93, 95
— — in sand culture, 41, 54, 55, 103-107, 156
— — in water culture, 14, 25, 54, 55, 103-107, 143
— — technique, 103-107, 143
— need of, 2
— purification of compounds of, 80, 81, 82, 102, 194-195
— ratio to other elements, 84, 108, 114
— in relation to
aeration, 24, 133
calcium supply, 107
crop, 103, 104, 105, 106, 107, 200
culture vessel, 14, 24, 101
light, 105, 106, 108, 159, 161
nitrogen supply, 95
nutrient composition, 104-108
pH, 95, 99, 102, 103, 105, 107, 108
phosphorus supply, 24, 99, 102, 103, 104, 105, 106
sand or water culture, 102, 104, 108
solution renewal, T19, 103
temperature, 160
— toxicity, 106
— sources of : chloride, 85, T19, 103-104, 105, 106, 188
— — citrate, 85, 86, T19, 103, 104 ,105, 106, 108, 188
— — in comparative trials, 104, 105, 106
— — humate, T19, 99, 102, 106, 107
— — magnetite, T19, 99, 102, 103, 190, see also Magnetite
— — miscellaneous compounds, T19, 102, 104, 105, 106, 107, 108
— — phosphates, 84, 85, T19, 101, 102, 103, 104, 105, 106, 107, 108
— — sulphates, 85, 86, T19, 101, 103, 104, 105, 106, 108
— — tartrate, 85, T19, 99, 103, 105
— valency, 95, 104, 105, 106, 107, 108
— See also Chlorosis
Irrigation, see Renewal of solution

K

Kale, 41, 57, 95
Keebush Sand purification apparatus, 175-177
Kentucky blue grass, 149, 163
Kok-saghyz, 112, 131, 151

L

Large-scale cultures ; materials, 132, 134, 136, 138, 143-147, 148, 172-200
— pumps and fittings, 145, 147
— sand culture
— — containers, 6, 8, 9, 17, 28, 129, 131, 132, 134, 136, 144-147
— — drainage, 4, 18, 19, 131, 144, 145, 146, 148
— — nutrient supply, 87, T19, 129, 131, 147
— — operation, 129, 131, 132, 134, 136, 138, 144-147
— — particle size, 6, 27, 28, 144, 145
— — reservoirs, 144-146, 148
— — retention of sand, 4, 19, 144
— — water consumption, 48, 145
— water culture
— — aeration, 144
— — containers, 12, 13, 144, 145, 147
— — nutrient supply, 87, 144
— — pH control, 100, 101, 102
— — surface area, 133, 145
Layout, see Design
Leaching, see Sand, Sand culture
Lead, 29, 57, 66, 80, 82, 147, 155
Legumes, 7, 11, 13, 24, 40, 48, 96, 114, 133, 149, 150, 152, 165, 200
see also individual crops
Lemna, advantages and limitations of, 157
— controlled environment for, 161
— growth in water cultures, 4, 21, 54, 55, 67, 68, 74, 77, T19, 115, 149, 154
Lemon, 112, 152, see also *Citrus*
Lettuce, 7, 9, 10, 12, 13, 38, 39, 52, 57, 67, 98, 107, 111, 112, 114, 115, 149, 150, 156, 157, 161, 198, 199, 200
Light, controlled, 149, 159-160, 161-162
— integrators, 163
— intensity, effect on growth, 159
— — in controlled environments, 149, 161, 162
— — under glass, 159
— photoperiod, 156, 159, 160
— quality, 162
— in relation to boron, 159
— — chlorosis, 105, 159
— — crop, 156
— — germination, 152
— — greenhouse design, 159
— — iron, 105, 159
— — magnesium, 141
— — manganese, 159
— — nitrogen, 159
— — potassium, 87, 159
— — temperature, 159, 160
— — zinc, 159
— source, 149, 162
— supplementary, 163
Lolium, 162, see also Ryegrass
Long Ashton Research Station, 6, 21, 29, 42, 46, 47, 49, 51, 55, 57, 58, 59, 60, 61, 79, 84, 87, 94, 96, 98, 110, 114, 153, 171, 187, 192, 193, 200
Lotus, 164
Lucerne, 12, 87, 151, 156, 165, 200
Lupin, 27, 96, 105, 112

M

Macronutrient, 1, 2, 157
Magnesium compounds, impurities in, 65, 66, 67
— — purification of, 70-82
— supply, exchangeable, 35, 36, 37, 38

Magnesium supply, in sand, 32, T7
— — in solution, 84-86, T19, 189, 190
Magnetite
— micronutrients in, 30, 103
— toxicity from, 30, 102, 103
— see also Iron
Maize, 3, 7, 9, 10, 11, 13, 36, 50, 51, 67, 86, 90, 91, 94, 95, 97, 98, 100, 101, 102, 103, 104, 105, 106, 107, 108, 111, 113, 147, 149, 150, 151, 156, 163, 165
Manganese contamination in container, 28, 21
— — nutrient reagents, 64-66
— — sand, 29, T7, 33
— — water, 43, 44, 45, 46, 47, 49, 50, 51, 59, 60, 185
— deficiency, 2, 111, 112, 113, 157
— — techniques, 17, 20, 31, 75, 156, 158
— need of, 2, 3
— normal levels, T19, 111, 112, 113, 189, 190
— removal of iron, molybdenum or zinc from, 79, 82, 195
— sources of, 41, 99, 112, 114
— tests for, 62, 67, 69, 78, 184, 185
— toxicity, excess of 48, 108, 112, 156, 190
Major element, see Macronutrient
Marchantia, 154
Mariotte's bottle for stills, 55, 56, 186, 187, see also Renewal of solution
Mason jars, 22, 135
Mercurial fungicides, 20
Metaphosphate, 99, 106
Methylcarbamate, 97
Micronutrients adsorbed, see Ion-exchange cultures
— A-Z treatments, 85, T19, 109, 110
— in bitumen, 18, 47, 172
— contamination, see Containers, Nutrient reagents, Sand, Water, and respective elements, e.g. Boron
— deficiencies of, 17, 18, 75, 80, 90, 108, 110, 111-114, 157, 158, 199
— definition of, 1
— in dust, 15, 158
— essential, 1, 3, 110-111, 158, 163
— excess, 111, 112-114
— in glaze, 21, 174
— heavy metals in, 66, 151
— normal levels, 85, 86, T19, 110-114, 189, 190
— in plastics, 19
— potential and miscellaneous, 1, 75, 76, 81, 108-111, 158, 163, 188
purification of compounds of, 70, 79, 80, 81, 82, 194-195
— removal of, see Glass, Nutrient reagents, Sand, Water
— relation to aeration, 94, 110, 133
— — nitrogen supply, 94, 97, 98, 110, 133
— — solution renewal, 117
— in seed, 163, 164
— sources of, 114, 115
— supplements, 37, 85, 86, T19, 108-110, 188, 189
— techniques, 152, 157-158, 170-200
Millet, T4, 27, 28, 40
Minor element, see Micronutrient
Mint, 2
Molybdenum, contamination of containers, 21, 158
— — nutrient reagents, 51, 65-66, 158
— — sand, 29, T7, 33, 158, 179
— — water, 45, 46, 47, 49, 50, 51, 52, 55, 59, 60, 158, 186, 187
— deficiency, 113, 114, 115, 151, 157
Molybdenum, deficiency, techniques, 17, 54, 55, 114, 151, 152, 156, 158, 179, 192-195, 199, 200
— excess, 108, 114
— need of, 4, 76, 98, 111, 165
— normal levels, T19, 113, 114, 189, 190, 191
— removal, see Nutrient reagents
— relation to nitrogen supply, 94, 111, 165
— tests for, by *A. niger*, 33, 49, 51, 67, 68, 71, 196
— — by chemical or physical methods, 68
Mustard, 11, 96, 157, 198
Mycorrhiza, 143

N

Nickel, 29, 65, 76, 80, 82, 103, 109, 110, 115, 188
Nitrate absorption, effect of age, 95, 139
— nutrient supply, 139
— of pH, 93, 94, 139
— effect on pH, 93, 100, 101
— — on potassium requirements, 87
— assimilation, effect of micronutrients on, 94
— compounds, impurities in, 65
— — purification of, 70-82, 191-193
— concentrations supplied, 86, 87, 88, T19, 189, 190, 191
— requirements in relation to
crop, 86-88
sand or water culture, 86-88
season, 94
Nitrification, see Ammonium, Urea
Nitrite assimilation, 97, 98
— effect of pH, 97
— toxicity, 97
Nitrogen compounds levels, 84-88, 118, 189, 190, 191
— See also respective compounds
Nutrient reagents
— impurities in relation to source, 64, 66, 67, 69
— — see also respective compounds of, e.g. Calcium
— purification methods, 69-83, 191-196
— — calcium nitrate preparation, 70, 71, 82, 192
— — comparative tests of, 67, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, T18
— — criteria of, 69, 79, 80, 195
— — filtration, 70, 191, 193
— removal of boron, 70, 71, 72, 76, 82, 158, 191, 192
— — cobalt, 71, 76, 78, 80, 81, 82, 83, 194, 195
— — copper, 70, 71, 72, 73, 74, 75, 76, 77, 78, 80, 81, 82, 83, 111, 158, 191, 193, 194, 195
— — gallium, 71, 72, 75, 76, 77, 79, 80, 81, 83, 194, 195
— — iron, 71, 73, 74, 75, 77, 81, 83, 191
— — manganese, 70, 71, 73, 74, 75, 76, 77, 81, 82, 83, 191, 194, 195
— — molybdenum, 71, 72, 73, 76, 77, 78, 79, 80, 81, 82, 83, 158, 192-193, 194, 195-196
— — zinc, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 158, 191, 194, 195
— — other metals, 70, 72, 73, 76, 79, 80, 81, 82, 83
— use of adsorption on charcoal, 71, 72, 73, 74, 82
— — adsorption and precipitation by alkaline-earths and phosphates, 71, 73-78, T18, 81, 83, 191
— — distillation, 71, 72
— — extraction, 71, 72, 75, 80-81, 82, 83, 191, 193, 194, 195
— — ion-exchange, 82
— — miscellaneous methods, 74, 75, 81, 82

Nutrient reagents *contd.*
— — use of precipitation including co-precipitation by organic reagents and sulphides, 73, 76, 78-80, T18, 83, 192-193, 194, 195, 196
— — recrystallisation, 69, 70, 75, T18, 81, 82, 158, 191, 192, 193
— tests for contamination by boron, 67, 68, 69, 196-197
— — iron, 196
— — manganese, 67, 69, 196
— — molybdenum, 65, 67, 68, 69, 76, 77, 79, T18, 196
— — zinc, 67, 68
— — see also Heavy metals, Diphenylthio-carbazone
— unpurified reagents, 64, 158
— use of for deficiency of boron, 66, 67
— — copper, 67, 68, 75
— — iron, 67, 68
— — manganese, 67, 75
— — molybdenum, 67, 68, T18
— — zinc, 67, 68, 75
Nutrient supply
— adsorbed nutrients, see Ion-exchange cultures
— application of solutions, see Renewal of solution
— balance and interactions, 88, 90, 92, 93, 115, 167, 168
— composition, 84-116, T19
— — examples, 3, 84-116, T19
— — four salt solutions, 3, 85, 86, 89, 93, T19
— — three salt solutions, 3, 88, 89, 90, T19
— concentration, 83, 84, 88, 89, 90, 91, T19, 155
— — limits, 86-88, 91
— — optimum, 86-88, 89, 90
— — relation to climate, 91
— — — crop, 86-88, 91, 198
— — — diffusion, 155
— — — environment, 162
— — — osmotic pressure, 3, 89, 90, 91, 92, T19
— — — rate of renewal, 86-88, 90, 92
— — — salt tolerance, 91, 92, T19, 118
— hydrogen ion concentration, see pH
— individual ion effects, 90, 92, 167, 168
— solid nutrients disadvantages, 40
— — uses, 32, 35, 40, 41, 102, 104
— — see also Iron, Magnetite
— stock solutions, 188, 189, 190
— terminology, 83
— toxic constituents in, 64, 115

O

Oak, 2, 141
Oat, 2, 7, 11, 13, T4, 27, 28, 35, 37, 40, 41, 75, 87, 92, 94, 96, 97, 98, 105, 108, 112, 113, 115, 127, 131, 133, 138, 156, 163, 164, 199
Oligo element, see Micronutrients
Onion, 7
Orange, 8, T19, 102, 112, 113, 118, 152
— see also Citrus
Orchid, T19
Organic growth supplements, ascorbic acid, 115, 116, 164
— — B vitamins, 115
— — requirements, 111, 115-116, 164
Osmotic pressure, see Nutrient supply (concentration, osmotic pressure, salt tolerance), Sodium
Oxamide, 97
Oxime nitrogen, 98
Oximino, acids, 98
Oxygen, see Aeration

P

Paint, see Aluminium, Bitumen
Paraffin wax, 18, 19, 20, 172
Parsnip, 198
Pea (*Pisum sativum*), 7, 12, 13, 40, 52, 57, 67, 70, 87, 91, 94, 96, 97, 98, 105, 107, 112, 113, 115, 149, 163, 164, 165, 198
Peach, 5, 8, 9, 18, 24, 31, 87, 88, 91, 95, T19, 100, 102, 112, 114, 118, 141, 143, 145
Pear, T19
Pecan, 8, 96, 112, 118, 143
Pelargonium, 14
Peptone, 97
Pest control methods, 150-151, 153, 156, 199, 200
pH changes in solution in relation to
nitrogen supply, 93, 94, 96, 100
— — root gradients, 40, 100
— — solution renewal, 100, 117
— effects on germination, 99
— — growth, 98-99, 102
— — iron availability, 99, 102, 144
— — nutrient solubility, 99
— — — uptake, 98, 99
— — roots, 98, 99
— injury, 40, 98, 99, 100, 101, 144, 155
— optima in relation to
— — crop, 99
— — nitrogen supply, 94, T19
— — season, 94
— production by acid, 50, 99
— — by alkali, 99, 100
— — by carbonates, 41
— — by phosphates, T19, 100, 101
— — miscellaneous, T19, 100, 101
— regulation, automatic, 101, 102
— — by buffers, 102
— — by nitrogen compounds, T19
— — by solution renewal, 101, 102
— values in nutrient solutions, T19, 98-102
Phomatotrichum omnivorum, 74
Phosphorus absorption, 41, 86, 99, 117, 119, 139
— compounds used, 40, 70, 71, T19, 99
— — impurities in, 64, 65
— — purification of, 70-82
— deficiency, 48, 87, 99, 157, 199
— normal levels, 84-87, 88, T19, 119, 189, 190
— relation to iron availability, 99, 119
Photoperiod, see Light
Pine, loblolly, 96
— white, T19
— seedlings, T19
Pineapple, 11, 12, 67, 86, 95, 96, T19, 99, 103, 111, 112, 113, 114, 122
Pinus strobus, 5, T19, 119
— *radiata*, 10, 11, 12, 152
Plastics, 17, 19, 47
Plum, 11, 76, 113, 143, 150
Polygonum persicaria, 2
Polyrhiza, 67, 149
Polythene, see Containers, Plastics
Potassium absorption, 86, 87, 139
— compounds, impurities in, 65
— — purification of, 70-82
— concentration in relation to crop, 86, 87
— — light, 87, T19
— — nitrogen supply, 87, 88
— — solution renewal, 86, 87
— deficiency, 31, 48, 157, 199

Potassium exchangeable, 35, 36, 37, 38, 39
— need of, 4
— normal levels, 84-88, T19, 189, 190
— excess, 87
Potato, 6, 40, 41, 105, 112, 149, 151, 156, 163, 164, 200
— propagation, 153, 154
— sand cultures, 5, 7, T4, 27, 28, 57
— water cultures, 2, 12, 13, T19, 135
Pots, see Containers
Precipitation of nutrients, 99, 188
Propagation, see Raising culture plants
Prune, 12, T19, 143
Pteridophyte cultures, 19
Purification methods, see Nutrient reagents, Sand, Water

Q

Quantitative methods, see Absorption, Design, Growth, Nutrient supply, Toxicity
Quartz, 2, 23, 51, 52, 54, 56

R

Radish, 9, 92, 156, 198
Radioactive isotopes, applications, 54, 69, 116-117
Rain water, see Water
Raising culture plants, clonal stocks, 152, 156
— conditioning treatments, 139, 140, 152
— grafting, 153
— rooting, 143, 152, 153, 154
— rootstocks, 6, 141, 143, 152, 153
— selection, 152, 198
— spores, etc., 154
— sowing, see Seed germination
— transplanting, 152, 198-199
— tubers, 152, 153, 198
Rape, 96, 98, 105, 149, 157
Raspberry, 12, 16, 150
Renewal of solution or water, 7-13, 48, 86, 87, 117-131
— frequency or rate, 2, 9-13, 87, 100, 101, 117, 118, 129, 131, 143, 144, 145, 155, 199
— methods, airlift, 124, 129, 131, 132, 134, 142
— — alternating solution treatments, 107, 119
— — automatic irrigation, 8, 102, 103, 127, 129, 131, 132, 134, 136, 138
— — capillary tube, 120, 123, 125
— — circulation and distribution, 124, 131, 132, 134, 136, 144
— — constant level cultures, 121, 122
— — continuous renewal and drip cultures, 3, 8, 86, 87, 100, 101, 108, 117, 119, 120-126, 127, 128, 129
— — intermittent application, 117, 118, 127, 129
— — Mariotte's bottle, 121, 123, 124, 125, 126, 127
— — metering pumps, 127
— — saucers, 118
— — sprayed or atomised, 106, 122, 124, 141, 142
— — sub-irrigation, 118, 131, 138, 144, 150
— — surface application, 118, 129, 131, 132, 134, 136, 144
— — tensiometers, 122, 124
— — wick cultures, 122
— relation to aeration, 14, 119, 123, 127, 129
— — concentration, 48, 86, 87, 90, 100, 118
— — crop, 87, 118
— — iron requirements, 95, 103, 131, 144
— — nitrogen supply, 95, 118
Renewal relation to micronutrient deficiency, 120
— — pH, 100, 101, 129
— — potassium supply, 86, 87, 124
Re-use of solution, 101, 119, 129
Resin linings for containers or water tanks, 20, 47
Revolving table, 131, 166
Rhizobium, 76
Rhododendron, 9, T19, 103, 143
Rice, 25, 48, 95, 96, T19, 99, 104, 105, 106, 108, 133, 163
Ricinus, 96
Root atmosphere, see Aeration
Root contact absorption, see Absorption, Ion-exchange cultures
Root rots, 98, 99, 131, 135, 151, 153
Rootstock, see Raising culture plants
Rose, T4, 27
Rothamsted, 43, 52, 67, 85, 100, 104, 145, 166
Rubidium, 93, 109
Rye, 10, 25, 96, 105, 156, 164
Rye grass, 28, 39, 40, 96, 98, 162, 163

S

Salt tolerance, see Nutrient supply
Sand, adsorption on, 32, 174, 179
— chemical analysis of, 29, 30, T7, 33, T18, 155, 179
— particle size, distribution, T4, 26-29
— — relation to container, T4, 27
— — — moisture capacity, 6, 28
— — — renewal of solution, T4, 27, 28, 119, 153
— — — rooting, 27
— purification methods, 31, 32, T18, 174-180
— — leaching, 31, 32, 174, 179, 180
— — omission of, 30, 171, 174
— — use of, 2, 30, 158, 179, 180
— source, 30, 31, T7, 33, T18, 174
— toxic constituents, 30
— see also Sand culture
Sand culture, advantages, limitations and scope, see Comparison, Technique
— aeration in, 4, 119, 127, 129, 133, 155
— drainage and retention of sand, 4-5, T4, 18, 19, 118, 144, 145, 150, 172, 173
— leaching, 118, 141, 155
— see also Aeration, Containers, Renewal of solution, Sand, Solid rooting media
Sealed cultures, see Aeration
Season, 94, 161
Seed, germination, 2, 3, 139, 140, 149, 150, 151-152, 153, 154, 165, 197, 198
— growth factors in, 115, 164
— mineral content, 163, 164
— pure line, see Crop
— removal of parts, 115, 140, 164
— sterilization, 147-149 ; see also Sterile cultures
— utlisation or depletion of reserves, 2, 163, 164
— weight, 164, 165
Selenium, 4
Silica compared with glass, 23
Silica gel, 40
Silicon deficiency tests and supplements, 1, 2, 4, 51, 52, T19, 108-110, 111
Silver, 29, 76, 80, 109
Sodium, beneficial effects, 1, 92, 93
— in nutrient solution, 91, 92, 93, 109, 189, 190
— relation to species, 91, 92
— see also Nutrient supply
Soil acidity, 156, 198

Solid rooting media, 2, 26, 29, 40, 150 ; see also Sand
Sorghum, 13, 87, 156
Soya bean, see Bean
Spectrographic tests, 67, 68, 69, 75, 76, 80, 115
Spirodella, 105
Split root experiments, 25
— see also Containers (multiple)
Spuren element, see Micronutrients
Squash, 12, 149
Sterile cultures, 25, 97, 98, 115, 140, 149-150
— see also Seed sterilization
Stirring by aeration, 10-13, 99, 101, 132, 140
— mechanical, 10-13, 101
— need for, 101, 117
Strawberry, 6, 7, 9, 12, 92, 95, T19, 98, 100, 101, 113, 118, 131, 150, 153
Sudan grass, 87, 151
Sugar beet, 28, 41, 47, 92, 95, 102, 105, 112, 156, 164, 198, 199, 200
Sugar cane, 25, 50, 131, 153
Sugars for microorganisms, impurities and purification, 68, 71, 72, 73, 80, 82
Sulphur, 43
Sunflower, 6, 7, 10, T19, 107, 165
Support of plants, 16-17, 173
— tubers, 16, 17
Swede, 41, 198, 199
Synthetic resins and lacquers, see Resin linings

T

Tafo, West African Cacao Research Institute, 143
Tea, 7, T19, 99, 107, 143, 152
Technique, choice of, 157-158
— comparison of sand and water cultures, see Comparison
— limits of, 158
— for micronutrient deficiencies, 158, 170-200 ; see also respective elements
— requirements, 157, 158, 171
Temperature, cold storage, 143
— controlled, 151, 152, 160, 161, 162
— effect on chlorosis, 160
— — growth in cultures, 160
— — nutrient uptake, 144, 160
— — roots, 160, 161
— — salt tolerance, 91, 160
— see also Light
Tensiometer, see Renewal of solution
Tetranitromethane, 97
Titanium, 29, 109, 110
Tobacco, 9, 12, 25, 50, 92, 96, 97, 98, 105, 112, 115, 133, 149, 150, 151
Tomato, 66, 67, 76, 91, 92, 98, 112, 115, 119, 156, 157, 160, 161, 168
— in sand culture, 7, 8, 9, 10, 11, T4, 27, 28, 37, 48, 50, 57, 87, 91, 95, T19, 97, 102, 112, 113, 114, 118, 131, 150, 198, 199, 200
— in ion-exchange culture, 37, 38, 39, 108
— in water culture, 3, 11, 12, 13, 25, 37, 70, 86, 88, 91, T19, 98, 100, 101, 107, 114, 133, 135, 144
Tourmaline, 29, 114
Toxicity in sand or water culture, 155
— estimation of, 145, 169
— from containers, 18, 19, 147
— from equipment, 46, 147
see also Ammonium compounds, Heavy metals, Magnetite, Micronutrients, Nitrite, pH, Water, and respective elements, e.g. Zinc
Trace element, see Micronutrients
Tradescantia, 96
Treatment notation, see Design of experiments
Tree cultures, containers, see Containers
— dormancy (cold storage), 143
— pruning, 141, 143, 153
see also respective crops, Large-scale cultures and Raising culture plants
Trimethylamine, 97
Trinidad, Imperial College of Tropical Agriculture, 143
Tropical crops, 157 ; see also Choice of crops and respective crops
Tryptophane, 98
Tung, 8, 57
Turnip, 50, 111, 112, 198

U

Ulex, 108
Urea assimilation, 97, 98
— nitrification, 97
Ureate, 97

V

Vanadium, 1, 4, 29, 50, 59, 65, 73, 76, 79, 81, 109, 110
Vanilla, 26
Variability, see Growth
Vetch, 2
Visual studies, 169, 171
Vitamin B, C., etc., see Organic growth factors

W

Walnut, 2, 141
Water consumption, 48, 147, 181
— contamination, see respective sources
— purification methods, activated carbon, 62
— — demineralisation, see Ion-exchange
— — distillation, cleaning stills, 57
— — — design of stills, 50-56, 186-187
— — — efficiency of stills, 51, 52
— — — operation of stills, 50, 51, 54, 55, 186-187
— electro-osmosis, 42
— ion-exchange apparatus, 57-59, 61, 181, 182, 183
— — efficiency, 57, 58, 60, 61, T18
— — operation, 61, 62, 63, 182-184
— — synthetic resins, 57, 59, 61, T18, 181
— tests for quality, 62, T18, 184-186
— sources, 42
— — distilled, analysis, 51-55, 57, 59, T18, 186, 187
— — — applications, 2, 51, 52, 158, 186
— — — toxicity of, 51, 52
— — ion-exchange, analysis, 57, 59, 60, 61, 62, T18, 185, 187
— — — applications, 57, 147, 158, 181, 187
— — — comparison with distilled water, 57, 62, T18
— — — toxicity tests, 57
— — rain, analysis, 42-46, T18
— — — applications, 2, 42, 48, 158, 181
— — — collection, 42, 48, 180
— — — purification, see Water, ion-exchange
— — — storage, 46, 47
— — — toxicity, 46, 48
— — river, 2, 42
— — spring, 2, 42

Water sources, tap, analysis, 48, 49, 50, T18
— — — applications, 2, 48, 50, 147, 158, 187
— — — purification, see Water distillation, electro-osmosis, ion-exchange
— — — toxicity of, 50
— — storage, see Water sources, rain
Water culture, see Aeration, Chlorosis, Comparison, Container, Iron, Large scale cultures, Nutrient supply, Renewal of solution, Stirring
Water logging, 118, 119, 133, 153
Wax, see Absorption measurement, Containers, Paraffin wax, Sealed cultures
Wheat, 3, 5, 7, 10, 11, 13, T4, 25, 50, 87, 92, 94, 95, 96, T19, 98, 99, 101, 104, 106, 149, 156, 165
Wick cultures, see Renewal of solution
Wood, see Containers

Z

Zeolites, see Ion-exchange cultures
Zinc contamination in
containers, 21
nutrient reagents, 64-66, 102, 103
sand, 29, 33
water, 45, 46, 47, 51, 57, 59, 60, 61, 186
— deficiency, 113, 143, 157
— — techniques, 17, 74, 75, 151, 152, 156, 158, 179, 191, 200
— need of, 3, 111
— normal levels, T19, 111, 113, 189, 190
— removal, see Nutrient reagents
— tests for, 67, 68, 78
— toxicity, 18, 42, 46, 102, 103, 108, 113, 155
— see also Bitumen, Containers, Water storage

A List of Publications of the Commonwealth Bureau of Horticulture and Plantation Crops

Horticultural Abstracts. A quarterly abstract journal of current literature on horticultural science and plantation crops. First issued 1931. Annual subscription rates on application

Author and Subject Index to Volumes I-X (1931-40) 25*s.* 0*d.*

Author and Subject Index to Volumes XI-XV (1941-45) 35*s.* 0*d.*

Author and Subject Index to Volumes XVI-XX (1946-50) *In Press*

Technical Communications

9. A REVIEW OF THE LITERATURE ON STOCK-SCION INCOMPATIBILITY IN FRUIT TREES, with particular reference to pome and stone fruits. G. K. Argles. (1937) 5*s.* 0*d.*

14. PROPAGATION BY CUTTINGS AND LAYERS. Recent work and its application, with special reference to pome and stone fruits. R. J. Garner. (1944) 3*s.* 6*d.*

15. SPRING FROST DAMAGE IN ORCHARDS AND ITS POSSIBLE PREVENTION. (1945) 1*s.* 6*d.*

16. FURTHER WORK ON PLANT INJECTION FOR DIAGNOSTIC AND CURATIVE PURPOSES. W. A. Roach and W. O. Roberts. (Reprint from Journal of Pomology). (1945) 1*s.* 6*d.*

17. CHEMICAL COMPOSITION OF PLANTS AS AN INDEX OF THEIR NUTRITIONAL STATUS. D. W. Goodall and F. G. Gregory. (1947) 9*s.* 0*d.*

18. FRUIT FALL AND ITS CONTROL BY SYNTHETIC GROWTH SUBSTANCES. M. C. Vyvyan. (1946) 3*s.* 6*d.*

19. SEED PRODUCTION OF EUROPEAN VEGETABLES IN THE TROPICS. A. G. G. Hill. (1948) 2*s.* 0*d.*

20. GROWTH SUBSTANCES AND THEIR PRACTICAL IMPORTANCE IN HORTICULTURE. H. L. Pearse. (1948) 12*s.* 6*d.*

21. RECENT ADVANCES IN FRUIT JUICE PRODUCTION. V. L. S. Charley and others. (1950) 15*s.* 0*d.*

Occasional Papers

2. EXPERIMENTAL DATA ON ORCHARD AND SMALL FRUIT MANURING. S. T. Antoshin. (1933) 1*s.* 0*d.*

6. HARICOT BEANS. G. St. Clair Feilden. (1941) 1*s.* 0*d.*

Agricultural Science Abstracts

THE COMMONWEALTH AGRICULTURAL BUREAUX PROVIDE A COMPREHENSIVE abstracting service in the agricultural sciences. A staff of over 100 scientists, translators and indexers produce some 30,000 abstracts annually, published in a series of journals which are obtainable by subscription.

The subjects are:

ANIMAL BREEDING AND GENETICS, ANIMAL HEALTH, ANIMAL NUTRITION, DAIRY SCIENCE, ENTOMOLOGY, FIELD CROPS, FORESTRY, FOREST PRODUCTS AND UTILIZATION, HELMINTH-PARASITOLOGY, HORTICULTURE AND PLANTATION CROPS, MYCOLOGY, PASTURES, PLANT BREEDING AND GENETICS, SOILS AND FERTILIZERS

From the wealth of material collected Occasional Publications are prepared consisting of reviews of published work, especially on topics of current interest, and descriptions of experimental practice. A full list of books will be sent on application to the Publishing Manager,

COMMONWEALTH AGRICULTURAL BUREAUX
FARNHAM ROYAL BUCKS ENGLAND